The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 18 APRIL, 1958 NUMBER 4
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Δ^4-3-KETOPREGNENE-20α-OL AND Δ^4-3-KETOPREGNENE-20β-OL, TWO NATURALLY OCCURRING METABOLITES OF PROGESTERONE. ISOLATION, IDENTIFICATION, BIOLOGIC ACTIVITY AND CONCENTRATION IN HUMAN TISSUES*

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ABSTRACT

1. Δ^4-3-Ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol have been isolated from human ripe follicles, corpora lutea, placentae and fat tissue.

2. Both compounds are metabolites of progesterone.

3. Both compounds have progestational activity by Hooker-Forbes and Clauberg tests and are therefore to be regarded as gestagens.

4. The relative concentrations of both compounds in the placenta in the second to the tenth month of pregnancy and in the ovary during the menstrual cycle and pregnancy have been quantitatively determined and compared to progesterone levels in these tissues.

5. Neither progesterone nor the Δ^4-3-ketopregnene-20-ol fraction could be

Received September 3, 1957.

* This investigation was supported by research grants from the Deutsche Forschungsgemeinschaft and from the National Institutes of Health, U. S. Public Health Service (RG-2342).

A brief report of these results was presented by the authors at the Laurentian Hormone Conference, September 2–9, 1956 (*Recent Progr. Hormone Res.* **13**: 379, 1957) and in *Klin. Wchnschr.* **35**: 143, 1957.

found in either uterine muscle or mucosa within the limits of the method used.

6. A compound was found in placental blood which is probably identical with Δ^4-3-ketopregnene-20-ol.

IT HAS been generally supposed that progesterone is the only naturally occurring substance with a primarily gestagenic effect. The present investigation was undertaken to determine whether other progestationally active compounds, hitherto unrecognized, occur in man.

The method of Hooker and Forbes for the bio-assay of progestin is sufficiently sensitive to determine the progestin content of 0.5 cc. of plasma (1). The method has been used to measure gestagenic activity in the plasma of women during the menstrual cycle and pregnancy (2–9). However, physico-chemical assays of plasma samples from arm-vein blood for progesterone yielded values which were appreciably lower than those obtained by the bioassay of Hooker and Forbes (10–16). For this reason it was assumed that other naturally occurring substances are also effective in the Hooker-Forbes procedure, even though rather extensive specificity tests (17, 18) had not disclosed such compounds.

Meanwhile, during studies on progesterone in human blood and tissues, Zander and von Münstermann (19) had isolated a new substance (compound X) from placentae, placental blood, ripe follicles, and corpora lutea. The substance was only present in extracts in association with progesterone. Proof that compound X must be directly involved in the metabolism of this hormone was derived from the following evidence (20). Progesterone and compound X are found in fatty tissue during the luteal phase of the cycle and during pregnancy, but neither the hormone nor compound X is found in fatty tissue after the menopause (21). If postmenopausal women are given large amounts of progesterone by injection, then one finds in the fat not only progesterone but compound X (20). The present study reports the isolation, identification, biologic activity, and concentration in human tissues under various physiologic conditions, of compound X and its isomer.

ISOLATION FROM TISSUES AND BLOOD

A standard procedure for isolation and quantitative estimation has been described (22, 23). After extraction and distribution between various solvents, progesterone and its metabolite, compound X, can be isolated by paper chromatography in a 70%-methanol:n-hexane system. In this system at 37° C., compound X has an R_f value of 0.38, whereas progesterone under the same conditions has an R_f value of 0.73. Originally it was assumed that a single new substance was involved. However, by further paper chromatography (Tables 1 and 2) the pooled fraction could be sepa-

TABLE 1. PAPER CHROMATOGRAPHIC BEHAVIOR OF COMPOUNDS X_1 AND X_2 RELATED TO DESOXYCORTICOSTERONE (R = 1.00)

Compound	Propylene glycol/ toluene (22° C.)	Formamide/ benzene-cyclohexane (1:1) (22° C.)	Bush B (22° C.)	Bush A (38° C.)
Desoxycorticosterone	1.00	1.00	1.00	1.00
Compound X_2 or Δ^4-3-keto-pregnene-20α-ol	0.88	1.50	1.17	1.41
Compound X_1 or Δ^4-3-keto-pregnene-20β-ol	0.99	1.59	1.29	1.88
Progesterone	1.28	1.86	1.66	4.78
11-Ketoprogesterone	—	1.35	1.09	1.23
17α-Hydroxyprogesterone	0.73	0.93	0.78	0.64
1-Dehydroprogesterone	—	1.84	1.41	2.50
Testosterone	0.64	1.04	0.79	1.17
Testosterone (17α)	0.74	1.23	0.93	1.47
Δ^4-Androstene-3,17-dione	1.18	1.71	1.49	1.86
Adrenosterone	1.07	0.89	0.75	0.45
Δ^4-11β-Hydroxyandrostene-3,17-dione	0.60	0.32	0.32	—

rated into 2 compounds which were called X_1 and X_2. Both show an ultraviolet absorption maximum at 240 mμ. Their position on the paper can readily be determined by ultraviolet photography.

IDENTIFICATION

The paper chromatographic behavior of compounds X_1 and X_2 in different solvent systems is summarized in Tables 1 and 2.

TABLE 2. PAPER CHROMATOGRAPHIC BEHAVIOR OF COMPOUNDS X_1 AND X_2 RELATED TO PROGESTERONE (R = 1.00)

Compound	Phenylcellosolve/ heptane	Formamide/ heptane
Progesterone	1.00	1.00
Δ^4-Androstene-3,17-dione	0.67	0.29
Compound X_2 or Δ^4-3-ketopregnene-20α-ol	0.45	0.29
— —Acetate	2.09	2.53
Compound X_1 or Δ^4-3-ketopregnene-20β-ol	0.67	0.50
— —Acetate	2.21	2.80

Identification of compound X_1 as Δ^4-3-ketopregnene-20β-ol

This substance had R_f values identical with those of Δ^4-3-ketopregnene-20β-ol. Ultraviolet absorption, NaOH fluorescence (yellow) and the infrared absorption spectra indicated a Δ^4-3-ketone. Both the reduction with blue tetrazolium or silver diamine and the Zimmermann reaction yielded negative results. The substance reacted with iodine to produce a brown color. From their properties and behavior in different paper chromatographic systems, hydroxyprogesterones, ketoprogesterones, hydroxyandrostenediones, and 17-ketosteroids or 17-hydroxysteroids could be practically excluded.

Acetylation of 20 μg. of X_1 with acetic anhydride in pyridine (forty-seven hours at 20° C.) yielded an acetate which chromatographed faster than free Δ^4-3-ketopregnene-20β-ol or progesterone in the Bush A and formamide/cyclohexane systems. The acetate was resistant to saponification under mild conditions with $KHCO_3$ in aqueous methanol (twenty hours at 20° C.). The polarity of X_1 and its acetate indicates strongly the presence of a single secondary hydroxyl group. This observation provided further support for the identity of the isolated compound with Δ^4-3-ketopregnene-20β-ol.

Oxidation of 34 μg. of compound X_1 with pyridine-chromic acid (24) resulted in a neutral product which had the same R_f value as progesterone in the Bush A system and produced identical color reactions.

Identification of compound X_2 as Δ^4-3-ketopregnene-20α-ol

This compound was identified as Δ^4-3-ketopregnene-20α-ol, in part by the methods used for the identication of compound X_1. The R_f value of the acetate was identical with that of authentic Δ^4-3-ketopregene-20α-ol-acetate. X_2 reacted on paper with iodine to produce a blue color, which is characteristic of the α-isomer (25). Oxidation of X_2 yielded a neutral product, which had the same R_f value as progesterone and which produced color reactions identical with those of progesterone. In addition, the oxidation product of compound X_2 on treatment for two hours with 0.1 ml. of a solution of 0.1 mole thiosemicarbazide in 0.01 mole HCl yielded a reaction product ($\lambda_{max.}$ 302 mμ) which chromatographed in the formamide/benzene system with an R_f value identical with that of the bisthiosemicarbazone of authentic progesterone. Finally the identification was completed by infrared analysis in potassium bromide pellets, comparing compound X_2 and Δ^4-3-ketopregnene-20α-ol.

BIOLOGIC TESTS

The two isomers of Δ^4-3-ketopregnene-20-ol have progestational activity in both the Hooker-Forbes test and the Clauberg test.

Hooker-Forbes test

A solution of compound X isolated from biologic material was prepared in sesame oil at a concentration of 24.9 μg. per ml. Chemical investigation of this fraction had shown that it consisted chiefly of Δ^4-3-ketopregnene-20β-ol. The solution was diluted progressively, and doses of 0.00005 to 0.0185 μg. were injected into one or both uterine horns of 28 ovariectomized CHI mice according to the standard procedure for the Hooker-Forbes assay (1). In the stromal nuclei of the endometrium the substance evoked an appearance identical with that produced by progesterone. The minimal effective dose for this substance in the CHI mouse was approximately 0.0001 μg. (as compared to 0.0002 μg. for progesterone).

Traces of impurities which had been separated by further chemical purification from the pooled fraction were also tested for possible activity in the Hooker-Forbes bioassay. They were inactive.

Synthetic Δ^4-3-ketopregnene-20β-ol was dissolved in sesame oil and injected in doses of 0.000025 to 0.0004 μg. into the uterine horns of 14 ovariectomized CHI mice. The synthetic compound evoked the same nuclear response as does progesterone. The minimal effective dose of the synthetic substance in the CHI mouse was 0.0001 μg.

Synthetic Δ^4-3-ketopregnene-20α-ol was similarly dissolved in sesame oil. Doses of 0.000045 to 0.0006 μg. were injected into one or both horns of 28 ovariectomized CHI mice. This synthetic compound evoked the same stromal nuclear response as do progesterone and the β isomer, although the end-point, *i.e.*, the least amount which would produce the positive response, was somewhat variable. The minimal effective dose of Δ^4-3-ketopregenene-20α-ol in the CHI mouse was approximately 0.0005 μg.

Clauberg test

The biologic activities of synthetic Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol were investigated with the Clauberg test[1] as modified by Miescher and Gasche (26). Castrated female rabbits were used for the bioassay. Beginning ten days after castration the animals received 10 μg. of estradiol daily for six days. After this pretreatment the Δ^4-3-ketopregnene-20-ol isomers were injected subcutaneously daily for five days. The transformation of the endometrial glands was used as the criterion for the biologic effect as described by Miescher and Gasche.

Both compounds demonstrated progestational activity; Δ^4-3-ketopregnene-20α-ol was one-half to one-third as effective as progesterone, and Δ^4-3-ketopregnene-20β-ol was one-fifth to one-tenth as effective.

[1] We are most grateful for the biologic assays carried out with this test by Dr. P. Desaulles, Ciba Ltd., Basel, Switzerland.

CONCENTRATIONS IN HUMAN TISSUES AND BLOOD

All of the following results were obtained by physico-chemical assay.

Placenta. Levels of the 2 isomers, and the corresponding progesterone concentrations, in 40 placentae between the second and tenth month of pregnancy are listed in Table 3. Concentrations of the 2 isomers were

TABLE 3. CONCENTRATION OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN PLACENTAE

No.	Month of preg.	μg. Progesterone/Gm. wet tissue	μg. Δ^4-3-Ketopregnene-20-ol/Gm. wet tissue	No.	Month of preg.	μg. Progesterone/Gm. wet tissue	μg. Δ^4-3-Ketopregnene-20-ol/Gm. wet tissue
1	II	5.44	1.83	21	VII	1.79	0.60
2	II	4.2	0.55	22	VII	2.40	0.37
3	II	5.75	0.94	23	IX	2.27	0.20
4	II	6.60	0.36	24	IX	2.28	0.31
5	III	3.59	0.67	25	IX	1.97	0.42
6	III	6.37	1.59	26	X	2.37	0.69
7	III	4.00	1.82	27	X	2.44	0.13
8	IV	4.00	0.29	28	X	2.34	0.13
9	IV	1.70	0.55	29	X	2.34	0.13
10	IV	0.94	0.08	30	X	2.35	0.29
11	V	2.79	0.86	31	X	2.13	0.12
12	V	3.36	0.62	32	X	1.18	0.31
13	V	3.04	0.40	33	X	2.04	0.34
14	V	1.50	0.26	34	X	2.45	0.44
15	V	2.20	0.42	35	X	2.42	0.29
16	V	1.75	0.25	36	X	1.44	0.11
17	VI	2.28	0.21	37	X	0.96	0.10
18	VI	4.23	0.46	38	X	0.47	0.30
19	VI	1.20	0.28	39	X	1.04	0.09
20	VII	1.68	0.29	40	X	2.51	0.38

not separately determined. The values indicate an average placental progesterone: Δ^4-3-ketopregnene-20-ol fraction ratio of 5.8:1.0.

The progesterone concentration in placental tissue during the second and third month of pregnancy is higher than in later months. The same appears to be true for the 2 progesterone metabolites.

In Figures 1 and 2 the total amounts of combined Δ^4-3-ketopregnene-20-ol and the values of Zander and von Münstermann (22) for total progesterone of 80 placentae between the second and tenth month of pregnancy are compared. The series of 80 placentae includes the 40 in which both compounds were estimated.

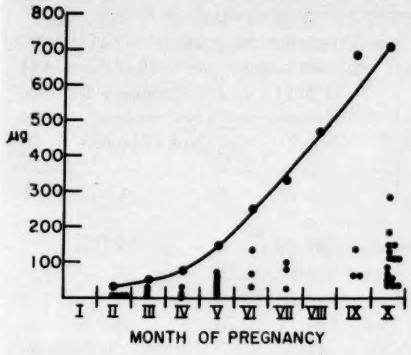

FIG. 1. Total amounts of combined Δ^4-3-ketopregnene-20-ol (small dots) in each of 40 placentae in the second to tenth month of pregnancy. The values are compared with the *average* values of progesterone (large dots on the line) in 80 placentae, as published earlier by Zander and von Münstermann (22).

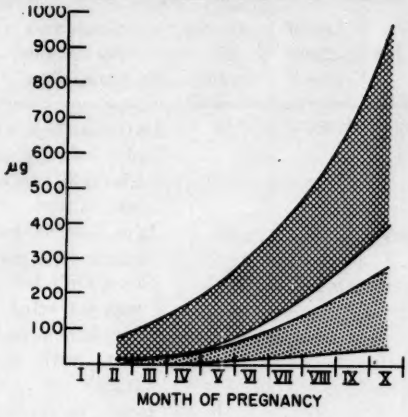

FIG. 2. Areas of distribution of the total amounts of combined Δ^4-3-ketopregnene-20-ol (lower area) and progesterone (upper area) in placentae in the second to tenth month of pregnancy.

To determine whether placentae contain both isomers, extracts of several placentae were pooled and chromatographed in the systems previously described. The results show that both isomers, but chiefly Δ^4-3-ketopregnene-20α-ol, are present in placentae. The ratio of α to β fraction is variable.

Ripe Graafian follicles. Results are summarized in Table 4 and Figures 3 and 4. The 7 follicles were obtained one or two days prior to ovulation. Progesterone was detected in all follicles. In 6 of them the combined Δ^4-3-ketopregnene-20-ol fraction was also found; separate determinations of the α and β fraction were not made.

Corpora lutea of the cycle. Results for 28 such corpora lutea are presented in Table 5 and in Figures 3 and 4. In 25 of these, the Δ^4-3-ketopregnene-20-ol fraction was present. The ratio of α to β fraction was variable.

Postmenstrual corpora lutea. Results are presented in Table 6 and in Figures 3 and 4. Only progesterone was detected in the corpus luteum of the third day of menstruation. No progestational material was found in older corpora lutea.

Corpora lutea of pregnancy. Results from 19 such corpora lutea are summarized in Table 7. In 12 of these cases the Δ^4-3-ketopregnene-20-ol fraction was detected. Individual measurements of the 2 isomers were not undertaken. Neither the concentration of progesterone nor the concentration of the Δ^4-3-ketopregnene-20-ol fraction in the first third of pregnancy

TABLE 4. LEVELS OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN RIPE FOLLICLES

No.	Age of patient (yrs.)	Day of cycle	Gross and histologic appearance	μg. Progesterone/ Gm. wet tissue or fluid	μg. Δ^4-3-Ketopregnene-20-ol/Gm. wet tissue or fluid
1	47	19	2.3 Gm. follicle wall plus 2.5 ml. fluid. About 36 hrs. before rupture	3.7	Not detected
2	28		Ripe follicle, ruptured at operation. Only follicle wall extracted	150.5	19.0
3	42	15	Ripe follicle (1.35 Gm. with contents)	17.1	Present; not enough to measure
4	27	10	Ready to rupture; 0.5 Gm. follicle wall plus 5.4 Gm. fluid	1.3	0.5
5*	28	14	*Idem.;* 6 Gm. tissue and fluid	8.2	Present; not enough to measure
6	50	19	Just before or during rupture (1 Gm. tissue)	11.0	Present; not enough to measure
7	41	13	Apparently just ruptured; 1.2 Gm. tissue	9.1	2.0

* Published earlier (15).

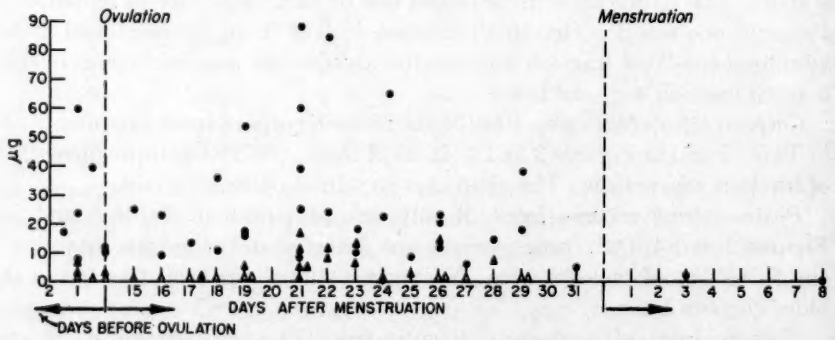

FIG. 3. Total amounts of combined Δ^4-3-ketopregnene-20-ol (triangles) and of progesterone (dots) in each of 7 follicles obtained one to two days before ovulation, and 32 corpora lutea of the cycle. The open triangles represent values which were too low for an exact quantitative determination.

TABLE 5. LEVELS OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN CORPORA LUTEA OF SECRETORY PHASE OF CYCLE

No.	Age of patient (yrs.)	Day of cycle	Histologic appearance	μg. Progesterone/Gm. wet tissue	μg. Δ^4-3-Ketopregnene-20-ol/Gm. wet tissue
1	43	15	Very early, soon after rupture	14.7	Present, not measurable
2	29	16	24–36 hrs. old	12.4	Present, not measurable
3	30	16	Young corpus luteum	12.1	Not detected
4	41	17	Not observed	10.5	Present, not measured
5	40	18	2 young c.l. in one ovary, apparently same age	a.25.5 b. 3.9	Present, not measurable
6	31	19	Well developed c.l. Early secretory mucosa	15.4	Present, not measurable
7	20	19	Well developed c.l. 4–6 days post-ovulation	8.0	Present, not measurable
8	31	19	Not observed	6.0	1.8
9	52	21	Well developed c.l. Early secretory mucosa	44.6	2.6
10	44	21	Well developed c.l.	4.6	0.9
11	22	21	Early secretory mucosa	44.1	4.6
12	33	21	Well developed c.l.	21.8	9.8
13	37	21	Well developed c.l.	13.9	6.8
14	34	22	Well developed c.l.	22.4	2.2
15	22	22	Not observed	15.3	7.8
16	31	23	Not observed	6.5	6.0
17	20	23	Well developed c.l.	12.3	9.9
18	46	24	Not observed	41.3	Present, not measurable
19	47	24	Not observed	15.3	Not detected
20	43	25	Mucosa 10 days post-ovulation	3.2	1.3
21	27	26	Secretory mucosa	13.1	Present, not measured
22	43	26	Premenstrual mucosa	9.9	Present, not measured
23	26	26	Not observed	7.9	5.0
24	41	27	Well developed c.l.	4.7	Present, not measured
25	30	28	Mucosa 2 days before menstruation	5.7	3.6
26	24	29	Premenstrual mucosa	12.9	Present, not measurable
27	46	29	Premenstrual mucosa	13.2	Present, not measurable
28	50	32	Premenstrual mucosa	4.4	Not detected

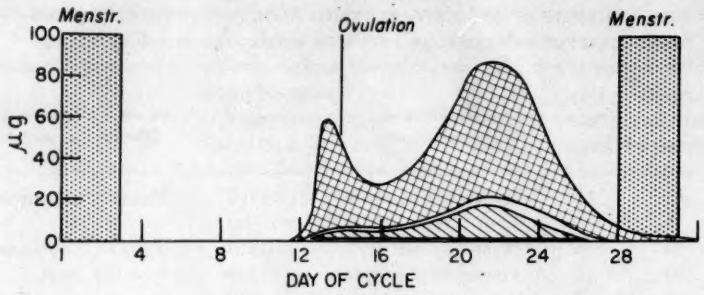

FIG. 4. Areas of distribution of the total amounts of combined Δ^4-3-ketopregnene-20-ol (lower area) and progesterone (upper area) in follicles and corpora lutea during the cycle.

was higher than the level of the corresponding substance during the secretory phase of the menstrual cycle. However, the majority of these corpora lutea of pregnancy were associated with pathologic conditions.

Blood. From placental blood of the tenth month of pregnancy a substance was isolated which had a maximal ultraviolet absorption at 241 mμ. Upon paper chromatography it showed the same behavior as the Δ^4-3-ketopregnene-20-ol fraction. The amount was too small for quantitative determination, although the progesterone in placental blood could be measured accurately.

TABLE 6. LEVELS OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN POSTMENSTRUAL CORPORA LUTEA

No.	Age of patient (yrs.)	Day of cycle	Histologic appearance	μg. Progesterone/Gm. wet tissue	μg. Δ^4-3-ketopregnene-20-ol/Gm. wet tissue
1	43	3	Regressing c.l. Some cells still seemed functional	4.4	Not detected
2	29	8	Postmenstrual c.l.	Not detected	Not detected
3	31	21	1 c.l. from each ovary; regressed, nonfunctional	Not detected	Not detected
4	47	2 mos. after last menst.; 1 mo. after radium radiation	2 nonfunctional c.l.	Not detected	Not detected

TABLE 7. LEVELS OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN CORPORA LUTEA OF PREGNANCY

No.	Month of preg.	Age of patient (yrs.)	Clinical diagnosis	μg. Progesterone/Gm. wet tissue	μg. Δ^4-3-Ketopregnene-20-ol/Gm. wet tissue
1	II	20	Extra-uterine preg.	49.8	Present; not measurable
2	II	20	Normal preg. Carcinoma of cervix	5.7	Not detected
3	II	24	Extra-uterine preg.	9.9	Present; not measurable
4	II	34	Extra-uterine preg.	Not detected	Not detected
5	II–III	30	Extra-uterine preg.	Present; not measurable	Not detected
6	II–III	34	Normal preg., interruption	4.9	Present; not measurable
7	III	35	Normal preg., interruption	Present; not measurable	Present; not measurable
8	III	30	Normal preg., interruption	21.7	Present; not measurable
9	III	32	Normal preg., interruption	8.0	1.45
10	III	45	Hydatid mole	3.1	Present; not measurable
11	III–IV	35	Extra-uterine preg.	8.2	Present; not measurable
12	III–IV	40	Extra-uterine preg.	11.5	Present; not measurable
13	IV	41	Extra-uterine preg.	6.8	Present; not measurable
14	IX	30	Placenta praevia; cesarean section	2.0	Not detected
15	X	31	Uterine inertia; cesarean section	6.7	Present; not measurable
16	X	27	Cesarean section	15.0	Not detected
17	X	40	Cesarean section	7.4	Not detected
18	X	35	Cesarean section	Not detected	Not detected
19	X	36	Cesarean section	3.6	Present; not measurable

TABLE 8. LEVELS OF Δ^4-3-KETOPREGNENE-20-OL (TOTAL FRACTION) AND OF PROGESTERONE IN THE FAT TISSUE OF POSTMENOPAUSAL WOMEN AFTER INJECTION OF PROGESTERONE

No.	Age of patient (yrs.)	Progesterone injected	μg. Progesterone/Gm. fat tissue	μg. Δ^4-3-Ketopregnene-20-ol/Gm. fat tissue
1	52	2 Gm. within 2 days before operation	2.0	1.2
2	44	2.5 Gm. within 3 days before operation	0.7	0.3

Neither of the Δ^4-3-ketopregnene-20-ol isomers could be detected in 93 samples of up to 50 ml. of plasma from peripheral blood collected from women during the menstrual cycle or pregnancy (16). The amounts, if any, of the α and β isomers in such samples are evidently too small to be revealed by physico-chemical assay.

Uterine muscle and decidua. The 2 isomers were not detected in the muscle or mucosa of the pregnant uterus. It has already been reported (22) that no progesterone was found in these tissues within the limits of the method used.

Fat tissue. The isolation of progesterone from human fat tissue has been reported by Kaufmann and Zander (21). In addition to progesterone being found in the fat tissue of 6 women in the tenth month of pregnancy, small amounts of a substance with ultraviolet absorption at 240 mμ were also seen. Upon paper chromatography this substance had the same R_f value as the Δ^4-3-ketopregnene-20-ol fraction. Further identification and quantitative determination of the material was not undertaken. In the fat tissue of 1 of 4 women, a substance with the behavior of the Δ^4-3-ketopregnene-20-ol fraction was isolated in the corpus luteum phase of the cycle. Neither progesterone nor the Δ^4-3-ketopregnene-20-ol fraction could be found in the fat tissue of 2 postmenopausal women.

Progesterone was injected prior to operation in 2 postmenopausal women. The results of the determinations on fat tissue are summarized in Table 8. In addition to the progesterone, the Δ^4-3-ketopregnene-20-ol fraction was found in rather high concentrations. In the first case the 2 isomers were not separated. In the second case about 90 per cent of the material was identified as Δ^4-3-ketopregnene-20α-ol. Upon paper chromatography the small residue had the same R_f value as Δ^4-3-ketopregnene-20β-ol. However, the amount was too small for further identification.

DISCUSSION

Butt *et al.* (10), Pearlman and Cerceo (27, 28), Zander and von Münstermann (19) and Jones *et al.* (29) have reported the isolation of several unknown steroids with the Δ^4-3-keto configuration from human placentae or placental blood. A high degree of activity in the Hooker-Forbes test was shown by at least 1 of the 7 compounds isolated by Jones *et al.* (29).

Forbes, Zander, Neher and Desaulles (30, 31) reported the identification of a substance isolated by Zander and von Münstermann (19) as a mixture of Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol. Independently, Salhanick *et al.* (32) announced that one of the compounds isolated by Jones *et al.* (29) was Δ^4-3-ketopregnene-20α-ol. Meanwhile, Short (33) reported that he had been able to isolate from a ketonic fraction of human placenta a substance with maximum ultraviolet absorption at 240 mμ and

with an R_f value like that of Δ^4-3-ketopregnene-20α-ol. The same author isolated Δ^4-3-ketopregnene-20β-ol from mare placentae.Hayano *et al.* (34) isolated Δ^4-3-ketopregnene-20β-ol after incubation of cattle corpora lutea with progesterone.

Berliner and Wiest (35), investigating the metabolism of progesterone-C^{14} in eviscerated rats, isolated a series of progesterone metabolites. One of the compounds was identified by Wiest (25) as Δ^4-3-ketopregnene-20α-ol.

The results available up to now indicate that Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol are metabolites of progesterone. Berliner and Wiest (35) have shown that in the rat the liver is not essential for the reduction of the C-20-carbonyl group to the C-20-hydroxyl group.

The reduction of the 20-keto group of progesterone to a secondary alcohol group in the α position has long been known. Pregnanediol isomers with a 20-hydroxyl group in the β position have not thus far been described for man; they may, theoretically, be expected on the basis of our findings. Allopregnanediol-3β,20β was isolated by Pearlman (36) from ox bile, and by Brooks (37) from pregnant mare urine. Pregnanediol-3α,20β was isolated from the bile of pregnant cattle by Pearlman (38).

Among the more highly oxidized steroids of the adrenal cortex, the naturally occurring 20α- and 20β-hydroxy derivatives have been known for some time. Stereochemical aspects of the reduction at C-20 in these steroids have been reviewed in detail by several authors (39, 40).

In view of the biologic activity of Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol in the Clauberg test and the Hooker-Forbes test, we feel that both compounds should be regarded as naturally occurring gestagens.

On the basis of analogies with other hormones, Butenandt and Schmidt (41) suggested as early as 1934 that compounds formed from progesterone by the reduction of its 20-carbonyl group to secondary alcohol groups should show biologic activity. They produced Δ^4-3-ketopregnene-20α-ol for the first time by synthesis, and subjected the compound to the Clauberg test. A dose of 1 mg. failed to elicit a detectable effect on the endometrium; tests with larger doses were not performed. As the results reported here show, they failed to confirm their hypothesis because the dosage was too low.

It was mentioned earlier that assay of progesterone levels in pregnancy plasma by physico-chemical methods yielded results appreciably lower than those obtained when comparable plasmas were subjected to the Hooker-Forbes bioassay. The discovery that the latter method detects not only progesterone but also Δ^4-3-ketopregnene-20α-ol and Δ^4-3-ketopregnene-20β-ol may help to explain the discrepancy, assuming that both

metabolites are present in the blood. In view of the magnitude of the discrepancy between results obtained by chemical assay and by the Hooker-Forbes test, the possibility exists that still other compounds in the blood may also show activity in this bioassay.

As with other hormones, the problem of different degrees of activity of the 2 progesterone metabolites in different species already presents itself. The α-isomer is one-half to one-third as active as progesterone in the rabbit by the Clauberg test. About the same difference between the 2 compounds appears in the Hooker-Forbes test with the CHI mouse when the minimal effective dose of progesterone (0.0002 μg.) and of the α compound (0.0005 μg.) are compared. However, the β isomer has an activity one-fifth to one-tenth that of progesterone in the Clauberg test but is twice as potent as progesterone in the Hooker-Forbes test (CHI mouse). The activity of the isomers in other species remains to be determined.

Recently, Zarrow *et al.* (42), using Rockland-Swiss mice, showed that 17α-hydroxyprogesterone has a high degree of activity in the Hooker-Forbes test, although not active in the Corner-Allen test. In our strain of mice (CHI), this compound yielded negative results in the Hooker-Forbes test (unpublished data). It thus appears that not only species but strain differences are involved in the biologic response to the compound.

The concentrations per gram of tissue, and the total amounts of progesterone in the human placenta in various months of pregnancy, were reported by Zander and von Münstermann (22). This work shows that the concentration of the Δ^4-3-ketopregnene-20-ol fraction essentially follows that of progesterone. The fact that these compounds are already present in the placenta in the second month of pregnancy is evidence that this organ has an early role in the production of gestagens.

Chemical isolation of progesterone from ripe human Graafian follicles and from corpora lutea of the menstrual cycle and pregnancy has been reported by Zander (15, 43). Forbes (3) and others have indicated that gestagens are present in blood prior to ovulation. The occurrence of progesterone and the Δ^4-3-ketopregnene-20-ol fraction in the ripe follicle is further evidence that gestagens are produced in the human ovary before ovulation. The high concentration may be due to storing of the steroids in the follicle immediately before its rupture.

The amounts in the corpora lutea of the cycle in relation to time agree with the known excretion of pregnanediol and with the histologic changes in the corpora lutea (44). We conclude, therefore, that the highest point of formation of progestationally effective substances in the corpus luteum appears to be between the seventh and eighth day following ovulation.

Acknowledgments

The authors express grateful thanks to the Director of the Universitäts-Frauenklinik, Köln, Germany, Dr. C. Kaufmann; to the Chairman of the Department of Anatomy, Yale University, Dr. W. U. Gardner; and to Dr. F. Gross and Dr. A. Wettstein, Ciba Ltd., Basel, for their continuing encouragement, their criticism and advice. We are also indebted to Dr. L. T. Samuels, Chairman of the Department of Biological Chemistry, University of Utah, for his pertinent criticisms of the manuscript. Gratitude is due Miss Rosemarie Eysell, Köln, and to Mrs. Ann Burrow, New Haven, for their skillful technical assistance.

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I^{131} METABOLISM IN THYROID SLICES OF PATIENTS WITH VARIOUS THYROIDAL DISORDERS*

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ABSTRACT

Slices of thyroid tissue, obtained at operation from euthyroid patients and from patients with Graves' disease, adenoma, carcinoma and several types of thyroiditis were studied in a gravity-flow system of incubation in a medium of constant composition containing I^{131}. The metabolism of I^{131} was expressed in terms of the clearance of I^{131} by the slice, tissue-medium (T/M) gradients, ability to bind iodine in organic form, and ability to discharge accumulated radioiodine in response to thiocyanate both in the unblocked state and after blocking with methimazole.

Slices of normal thyroid tissue had similar clearance values (0.03 ml./min./Gm.) and T/M ratios (3 to 4) whether organic binding was permitted or abolished. Only half of the accumulated I^{131} was protein-bound when binding was allowed, and the remaining I^{131} was dischargeable by thiocyanate. When Lugol's solution had been administered preoperatively, the above values were reduced to 50 and 75 per cent. The clearance by slices of exophthalmic goiters was tenfold, and the T/M ratios sixfold greater than those of normal tissue. The protein binding was one third that of normal tissue. Thiocyanate induced marked discharge of I^{131} from either the blocked or the unblocked slice. The suppression due to preoperative administration of Lugol's solution was proportionally the same as noted for normal slices, but the suppressed exophthalmic-goiter tissue still metabolized I^{131} in amounts five to eight times greater than did the suppressed normal tissue.

Adenomatous tissue generally metabolized I^{131} in the same manner as normal thyroid tissue. However, in 5 cases, thiocyanate did not discharge I^{131} from the unblocked slice, and in 3 cases thiocyanate was ineffective in both the blocked and the unblocked slice. Three of the 4 thyroid carcinomas metabolized I^{131} in the same manner as normal tissue; in the fourth, however, the accumulation of I^{131} followed the pattern observed in Graves' disease, though less intense. Thiocyanate did not discharge I^{131} from slices of the first 3 carcinomas, but did so readily from the fourth or "hyperfunctioning" tissue. Slices from cases of Hashi-

Received July 22, 1957.

* Abridgment of portion of thesis submitted by Dr. Schumacher to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medicine.

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moto's thyroiditis were "hyperfunctional" with respect to I^{131} metabolism, but less so than slices from cases of Graves' disease. Thiocyanate induced substantial losses of I^{131} from either blocked or unblocked tissue. Tissue from 2 cases of granulomatous thyroiditis showed values for I^{131} metabolism like those of normal tissue, except for the extremely low content of protein-bound I^{131}.

IN a previous report from this laboratory (1), a method for incubating thyroid slices in a medium of constant composition was described and the metabolism of I^{131} in slices of normal thyroid tissue in such a system was determined. The present report is an extension of this method of study to thyroid tissue of patients with various disorders of the thyroid.

MATERIALS AND METHODS

1. *Experimental technic.* Thyroid tissue was obtained from 45 patients who were undergoing thyroidectomy as prescribed treatment for adenoma, carcinoma, or thyroiditis. Tissue slices were prepared and incubated in the same manner as described previously (1). The incubating medium usually contained 0.04 μc. of I^{131} per milliliter, although occasionally greater amounts of radioiodide (to minimize analytic error) were employed when I^{131} had been given to the patient preoperatively for diagnostic purposes. Three separate flasks containing incubating fluid were prepared. Each flask contained the basic incubating medium of Krebs-Henseleit solution and radioiodide. The first flask (flask 1) contained only the basic medium, the second flask (flask 2) contained 10^{-3} *M* methimazole (Tapazole) in the basic medium, and the third flask (flask 3) contained 10^{-3} *M* methimazole and 0.2 *M* sodium thiocyanate in the basic medium. Each experiment employed 4 tissue chambers, each chamber containing 2 thyroid slices. Two of the chambers were perfused with the basic medium (flask 1), and 2 chambers received the basic medium containing the methimazole (flask 2) for one hundred minutes. At the end of this time, 1 chamber from each of the two sets was removed for analysis of the thyroid slices. The remaining 2 chambers were then connected for eighty minutes to flask 3, containing the thiocyanate. The perfusion rate in all experiments was 0.5 ml. per minute.

An end-window sodium iodide (thallium-activated) scintillation counter attached to a Model 1078 Multiscaler was used for serial determinations of the radioiodine uptake of the slices during the course of incubation. The uptake of I^{131} by unblocked slices was shown in preliminary experiments to be linear over an incubation period of eight hours. In blocked slices, it was assumed for the sake of simplicity that the uptake was also linear over the short interval of one hundred minutes.

2. *Method of calculation.* Several calculations of the raw data obtained as total counts over the chambers during the course of incubation were necessary to express the results on some common basis. These calculations, a full example of which is given in Table 1, follow: The total counts obtained serially over a chamber during the course of incubation were plotted against time (Fig. 1). The zero intercept was determined and subtracted from the total counts at each time "t" during incubation (column 1, Table 1). This subtraction yielded the net counts over the slices at various intervals. From the net counts per gram of tissue and from a similar count with the same geometry of 1 ml. of incubating fluid in the chamber, T/M (tissue/medium) ratios were determined for the entire incubation period. These T/M ratios were plotted to depict on a common basis the accumulation and discharge of I^{131}. The T/M ratio curves were used only to

TABLE 1. CALCULATION OF VALUES PLOTTED IN FIGURE 1: NORMAL THYROID TISSUE (BLOCKED)

	Time	A Total count	B Net count	C T/M	
I	0	316	0	0	Counts per cc. of medium (end-window) =240 cts./s./cc.
	4	321	5	0.08	Counts per cc. of medium (well counter)
	12	331	15	0.26	=1,600 cts./s./cc.
	22	338	22	0.39	Weight of thyroid slices =0.240 Gm.
	36	351	35	0.62	Counts of thyroid slice in well counter =274/s.
	48	368	52	0.92	
	66	388	72	1.27	
	76	397	81	1.43	
	86	402	86	1.51	
	96	410	94	1.65	
	102	416	100	1.76	
II Average	[55]	[372]	[56]	[0.99]	
	105	(0.2 *M* SCN medium)			
	107	418	102	1.80	
	120	404	88	1.55	
	148	387	71	1.25	
	168	385	69	1.21	
	176	385	69	1.21	

III $$T/M = \frac{\text{Net counts of slices (end-window)}}{\text{Cts./cc. of medium} \times \text{wt. of slice in Gm. (end-window)}} = \frac{5}{(240 \times 0.240)} = 0.08$$

IV $$T/M_{t+SCN} = \frac{\text{Cts. of slice in well counter}}{(\text{Cts./cc. of medium}) \times (\text{wt. of slice in Gm.})} = \frac{274}{1{,}600 \times 0.240} = 0.7$$

[well counter]

V $$\text{Rate} = \frac{(\text{Average cts.} - \text{cts. at } t = 0)}{(\text{Average time} - 0)} = \frac{372 - 316}{55} = 1.02 \text{ cts./s./min.}$$

VI Rate/Gm. = Rate/wt. of slice in Gm. = 1.02/0.240 = 4.2 cts./s./min./Gm.

VII $$\text{Clearance}_t = \frac{\text{Rate/Gm.}}{\text{Cts./cc. of medium}} = 4.2/240 = 0.02 \text{ cc./min./Gm.}$$

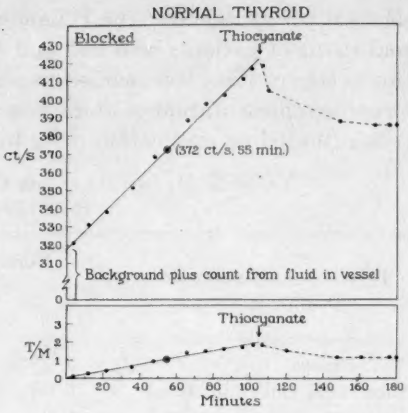

FIG. 1. Details of analysis of uptake curve (normal thyroid tissue, blocked with methimazole). The upper curve is plotted directly from the points obtained during the course of the incubation. These (total counts/second) are recorded in column A of Table 1. The net counts (column B) are calculated by subtracting the intercept at time zero (316 counts/second) from the experimental values. These values are then converted to T/M values by formula III of Table 1 and are recorded in column C, T/M.

sort the data into various groups (see later), and only a few examples of these values are presented in graphic form.

Although the T/M ratios thus obtained served as a guide for determining the type of behavior of the thyroid slice, a better comparison of the metabolism of I^{131} in different slices was afforded by the determination of clearance. The counts were averaged and the rate of uptake of I^{131} was calculated as described (Table 1) and converted to rate per gram by appropriate correction using the weight of the slices measured at the end of the experiment. Since this value, net count per gram per minute, is dependent on the I^{131} concentration of the medium, which could vary slightly from experiment to experiment and which in some experiments was purposely greatly altered, the rates were converted into clearance values. Thus a parameter of thyroid-slice function was attained which compensated for differences in weights of slices and concentrations of I^{131} in the medium.

Another set of T/M ratios was calculated from analyses made at the end of the incubation period, and these are the T/M values presented in Tables 2 through 6. These T/M ratios were obtained for the slices in the 2 chambers removed at one hundred minutes, and for the 2 at one hundred and eighty minutes. They were determined by counting the weighed slices and 1 ml. of incubating medium in the well-type scintillation counter. Because the well counter is much more sensitive than the end-window counter, more accurate determinations of T/M could be made when the uptake by the slice was low. Although the T/M ratios determined in this fashion were more precise, they were not useful for comparison of slices before and after exposure to thiocyanate because of the difference in incubation time. Thus determination of T/M values by two methods was necessary. One method provided information during the incubation of the slice, and the other provided values under conditions of low iodine accumulation.

The final analysis on each slice was the determination of the tissue protein-bound radioiodine (TPI) in both the blocked and the unblocked system. The TPI was determined in the usual manner by means of trichloroacetic-acid precipitation of suitably prepared homogenates of slices. The TPI of the blocked slice was always negligible; hence it was not recorded.

RESULTS

1. *Normal and hyperplastic control tissue.* Since it was occasionally necessary to use slices of thyroids from patients who had received Lugol's

solution preoperatively, the behavior of normal and of hyperplastic thyroid tissue of patients who had and who had not been given Lugol's solution preoperatively was studied to provide base line information (Table 2). Graphic representation of uptake curves for both normal and hyperplastic tissue (no iodine medication prior to thyroidectomy in either instance) is

TABLE 2. MEAN VALUES FOR PARAMETERS OF I^{131} METABOLISM IN THYROID SLICES*

Previous medication with Lugol's solution	Normal tissue		Hyperplastic tissue	
	No	Yes	No	Yes
No. of cases	2	3	1	6
$Clearance_p$ (ml./min./Gm.)	0.03	0.01	0.30	0.08
$Clearance_t$ (ml./min./Gm.)	0.03	0.01	0.22	0.08
T/M_p	4.0	1.0	25.0	4.6
T/M_t	3.0	0.7	17.0	3.4
T/M_{p+SCN}	2.4	0.9	1.6	1.0
T/M_{t+SCN}	0.8	0.8	0.7	0.8
TPI, per cent	50	23	17	10

* Abbreviations in this and all subsequent tables are:
$Clearance_p$ = clearance of unblocked slice.
$Clearance_t$ = clearance of blocked slice.
T/M_p = tissue/medium ratio, unblocked slice.
T/M_t = tissue/medium ratio, blocked slice.
T/M_{p+SCN} = tissue/medium ratio in unblocked slice after thiocyanate.
T/M_{t+SCN} = tissue/medium ratio in blocked slice after thiocyanate.
TPI = tissue protein-bound iodine content.

given in Figures 2 and 3 respectively. These data show that the clearance values and the T/M ratios are similar whether protein binding of I^{131} is allowed or abolished. In slices of the normal thyroid, a greater discharge of I^{131} following addition of thiocyanate occurs in the blocked tissue rather than in the tissue which is binding I^{131} organically. Analysis of the slice disclosed that half of the I^{131} in the normal unblocked slice is protein-bound, which is reflected in the smaller discharge after addition of thiocyanate in unblocked tissue. Medication with iodine prior to operation had a depressing effect on protein binding of the unblocked slice and on the clearance values and I^{131} gradients of both the blocked and the unblocked slice.

The clearance values were eight to ten times higher in hyperplastic tissue than in normal tissue. This is also reflected by the higher T/M ratios. As in normal tissue, protein binding had little influence on the I^{131} gradients established between tissue and medium. Both clearance and gradient values were markedly depressed by the preoperative administra-

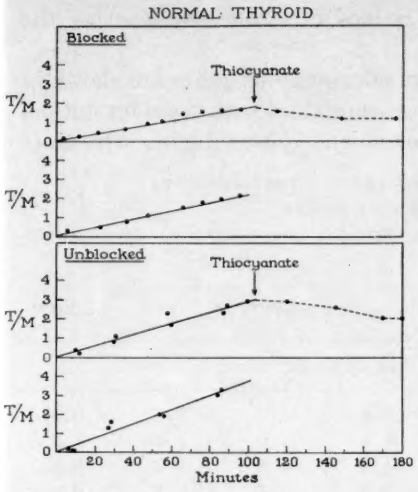

FIG. 2. T/M curves obtained from slices of normal thyroid tissue. Four curves are shown, 2 with methimazole (blocked), and 2 without methimazole (unblocked). The arrows indicate the addition of thiocyanate. There is demonstrable discharge in both the blocked and unblocked slices after addition of thiocyanate.

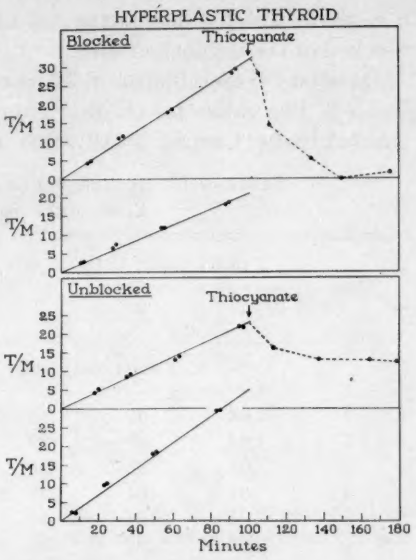

FIG. 3. T/M curves obtained from slices of hyperplastic thyroid tissue. Note that the scale of these curves is five times that of the curves in Figure 2. The discharge of I^{131} from both the blocked and the unblocked slices was large.

tion of Lugol's solution. Hyperplastic tissue bound I^{131} poorly (unless it is presumed that a low TPI is due to very rapid secretion of TPI into the medium). The data obtained by direct measurement of TPI agreed with the low T/M value of the unblocked hyperplastic slice after thiocyanate discharge. Lugol's solution depressed the capacity of the hyperplastic slice to bind I^{131} organically. Although the reduction was not as great after iodine medication in hyperplastic tissue as it was in normal tissue, the already low protein-binding capacity was even further reduced. Despite the marked inhibiting effect of Lugol's solution on the clearance values, the clearance of I^{131} by hyperplastic tissue was still eight times greater than that of normal tissue after similar medication.

2. *Adenomatous tissue.* Adenomatous tissue was obtained in the course of thyroidectomy from 30 patients. Of these, 23 had not been given iodine. On the basis of the T/M curves calculated during the course of the incubation, these cases (except for Case 15) were separated into three main groups: (a) 14 cases in which I^{131} metabolism was normal, (b) 5 cases in which thiocyanate did not discharge I^{131} from the unblocked slice, and (c)

3 cases in which thiocyanate did not induce I^{131} discharge in either the blocked or the unblocked slice.

Data on I^{131} metabolism in 22 cases of adenomatous goiter are shown in Table 3. The values for the first group are consistent with those for normal thyroid tissue. Certain T/M ratios (Cases 5 and 7) were higher after thio-

TABLE 3. I^{131} METABOLISM IN ADENOMATOUS THYROID TISSUE: NO PRETREATMENT WITH IODINE

Case	Clearance		TPI, %	T/M			
	p	t		p	p+SCN	t	t+SCN
A. *Values comparable to those of normal tissue*							
1	.03	.03	50	2.5	1.9	2.6	0.9
2	.03	.02	29	1.6	1.0	1.1	1.0
3	.05	.03	58	1.9	1.5	1.0	0.8
4	.04	.04	49	2.0	1.5	2.2	0.9
5	.05	.04	51	2.1	2.9	3.1	0.9
6	.03	.02	61	2.0	1.0	0.8	0.8
7	.11	.08	63	5.7	6.8	6.3	1.5
8	.06	.07	34	5.2	1.2	6.5	0.8
9	.04	.03	57	2.4	1.7	2.2	0.8
10	.04	.02	36	3.0	2.2	1.5	0.7
11	.03	.04	15	2.3	1.1	2.7	0.7
12	.03	.02	37	1.9	1.1	1.8	0.8
13	.04	.02	52	2.4	0.9	1.6	0.6
14	.03	.02	16	1.2	1.2	1.2	0.9
B. *No discharge of I^{131} in unblocked state after SCN*							
16	.02	.02	22	0.8	0.8	0.7	1.0
17	.02	.02	25	1.2	1.0	1.0	1.0
18	.04	.03	49	2.4	1.3	2.3	—
19	.07	.05	92	7.5	4.5	3.8	0.9
20	.07	.03	60	4.5	3.2	3.2	1.0
C. *No discharge of I^{131} in either blocked or unblocked state after SCN*							
21	.02	.01	65	1.5	1.5	0.5	0.5
22	.03	.01	27	0.8	1.4	0.7	0.7
23	.02	.02	48	0.9	0.9	1.2	0.9

cyanate than before. As explained under "Methods," these T/M ratios were determined on different slices and at different times of exposure. Singly, they are not to be compared with one another. Collectively, however, these T/M ratios are in agreement with those obtained serially

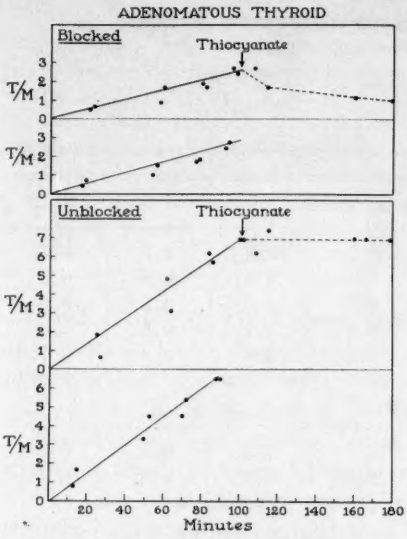

FIG. 4. (Case 19.) Adenomatous tissue, showing lack of discharging effect of thiocyanate in unblocked slices.

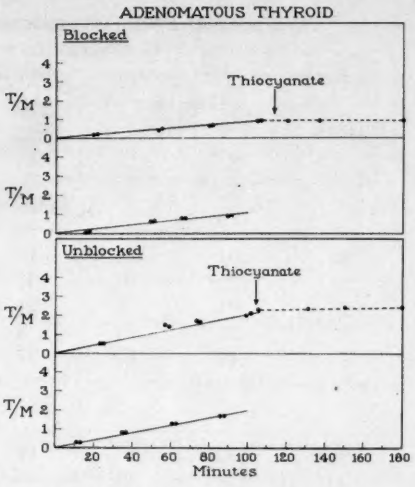

FIG. 5. (Case 21.) Adenomatous tissue, showing no effect of thiocyanate in either unblocked or blocked slices.

throughout the incubation. In any case, whether or not discharge occurred after addition of thiocyanate, was not decided from these T/M values, but rather on the basis of the gradient curves shown in the figures.

In 1 patient (Case 15), not included in Table 3, the lesion was a toxic adenoma and in this instance the adenoma was compared with the non-adenomatous (that is, normal) tissue. The clearance of the "hot" nodule was twice that of the normal tissue and its protein binding half that of normal.

In 5 cases the radioiodine was not discharged after perfusion of the unblocked slice with thiocyanate. An example of this behavior is shown in Figure 4 and the detailed data are listed in Table 3 (Cases 16–20). The clearance of radioiodide in these cases was normal, whether or not protein binding was blocked. However, the percentage of protein-bound radioiodine at the end of the incubation of the unblocked slice varied from low (22 per cent) to high (92 per cent). The patients in this group were euthyroid.

In 3 patients (Cases 21–23), thiocyanate did not effect a discharge of radioiodine in either the blocked or the unblocked slices (Fig. 5). The clearance values were normal or slightly low, and the protein binding high.

Table 4 shows the remaining 7 cases of adenomatous goiter. Iodine was

TABLE 4. I^{131} METABOLISM IN ADENOMATOUS THYROID TISSUE: IODINE GIVEN PREOPERATIVELY

Case	Clearance		TPI, %	T/M			
	p	t		p	p+SCN	t	t+SCN
A. *Iodine solution, strong*							
24	.01	.01	17	0.7	0.9	0.7	0.7
25	.02	.01	37	1.4	0.8	0.8	0.8
26	.01	.01	24	0.7	0.6	0.5	0.5
27	.01	.01	8	0.4	0.7	0.5	0.6
28	.02	.02	17	1.0	0.8	0.8	0.7
B. *Telepaque*							
29	.02	.01	60	1.2	1.3	0.8	0.6
30	.01	.01	9	0.8	0.7	0.6	0.5

given to these patients prior to operation. Five of them (Cases 24–28) were treated with Lugol's solution as a preoperative medication, and 2 (Cases 29 and 30) had received gall-bladder dye (Telepaque) for other diagnostic purposes some time previous to operation. Data on I^{131} metabolism in these cases are to be compared with those obtained in normal tissue under the influence of Lugol's solution. The clearance values were not different from those of normal thyroid tissue exposed previously to iodine therapy. The protein binding of I^{131} was low, as were the iodine gradients. Because of the low clearance values and T/M ratios, the alteration following sodium thiocyanate was of minor proportion (Cases 24–28), but discharge was nevertheless evident. However, Cases 29 and 30, in which Telepaque had been ingested, showed flat curves after the addition of thiocyanate.

3. *Carcinomatous tissue.* Carcinomatous thyroid tissue was available from only 4 patients (Table 5), and only small amounts of tissue were obtained, precluding complete analysis. In Cases 31 through 34 the patients had not received any iodine therapy prior to operation. In the first 3 of these, the clearance values were lower than normal, indicating only small uptakes of radioiodine. The protein binding was about half normal, and the T/M ratios, whether in the blocked or unblocked state, were also low. No discharge after thiocyanate occurred. In the fourth case the tissue behaved like hyperplastic tissue, showing high clearance values, low protein binding, and substantial I^{131} gradients.

4. *Thyroiditis.* Twelve cases of thyroiditis, of which 6 were classified as Hashimoto's, 3 as granulomatous and 3 as nonspecific thyroiditis, were

TABLE 5. I^{131} METABOLISM IN CARCINOMATOUS THYROID TISSUE

Case	Clearance		TPI, %	T/M			
	p	t		p	p+SCN	t	t+SCN
31	.01	.01	16	0.7	0.8	0.7	0.7
32	.01	.01	27	1.0	0.9	0.5	0.5
33	.02	—	29	2.1	—	—	—
34	.15	.11	27	13.4	—	12.8	0.7

studied (Table 6, which does not list 1 of the cases of granulomatous thyroiditis). These diagnoses were established by both the clinical characteristics and the pathologic examination (Dr. L. B. Woolner) of slices adjacent to the tissue studied.

The clearances of either blocked or unblocked slices in cases of Hashimoto's thyroiditis (Cases 35–40) were much greater than normal, although not as high as in slices of tissue from cases of Graves' disease. Also, the protein-bound iodine content was low and the discharge of radioiodide after exposure to thiocyanate was substantial. In Case 40, analyses were

TABLE 6. I^{131} METABOLISM IN THYROIDITIS

Case	Clearance		TPI, %	T/M			
	p	t		p	p+SCN	t	t+SCN
A. *Hashimoto's thyroiditis*							
35	.08	.07	23	9.2	1.7	5.4	0.9
36	.08	.08	21	7.5	1.5	6.2	0.9
37	.07	.06	12	3.6	1.4	4.4	0.9
38	.09	.10	12	5.3	1.4	7.7	1.1
39	.08	.10	20	6.4	1.4	8.1	1.5
40a	.04	—	26	2.6	1.0	—	—
40b	.02	—	13	0.7	0.7	—	—
B. *Granulomatous thyroiditis*							
41	.01	.01	—	—	—	—	—
42	.03	.04	4	2.9	0.7	2.9	0.6
C. *Nonspecific thyroiditis*							
43	.04	.02	17	1.5	1.1	1.7	0.8
44	.14	.13	66	3.6	2.1	4.1	1.2
45	.02	.01	26	1.4	1.1	1.3	0.9

made in 2 histologically distinct parts of the tissue removed at operation. One area (40a) was infiltrated heavily with lymphocytes but had intact follicles. The other area (40b) was characterized by an invasion of fibrous tissue with disrupted follicles. The former area, characterized by intact follicles, had a higher protein-bound iodine content than the latter.

There were 2 cases of granulomatous thyroiditis. In Case 41 the patient had received an I^{131} tracer and Lugol's solution preoperatively, making complete analysis impossible. The low clearance was probably due to the prior iodine medication. Of interest was the fact that 96 per cent of the radioiodine (from the preoperative tracer) in the granulomatous area was iodide as determined by column chromatography, whereas the uninvolved area had a normal distribution of radioiodine among the amino-acid constituents (Owen and McConahey (2)). In Case 42 the patient had had no medication preoperatively, and clearance values were normal. However, the protein-bound iodine value was extremely low. Both blocked and unblocked slices showed discharge after thiocyanate, and the gradients established did not differ from those of normal tissue. In another case of granulomatous thyroiditis, not listed in Table 6, analysis was performed for I^{131} (from the preoperative tracer) in two different portions of the tissue. The severely involved portion had a labeled iodide content of 71 per cent, whereas the histologically normal tissue had a labeled iodide content of 8 per cent.

Three cases of nonspecific thyroiditis are included in Table 6. The surgical specimen in Case 43 resembled a colloid goiter. Except for the low protein binding and the low iodine gradients, the slices from this specimen behaved like normal tissue. Case 44 showed nonspecific thyroiditis in the nonadenomatous portions of a nodular goiter. The clearance values were high, like those in cases of Hashimoto's thyroiditis, but the protein binding was also high, unlike that of Hashimoto's thyroiditis. In the last case (Case 45), a gall-bladder dye had been administered several days before operation. All values resembled those for normal thyroid tissue which had been exposed to iodine *in vivo*.

COMMENT

The present study was undertaken to survey generally the behavior of slices of abnormal thyroid tissue with respect to the metabolism of I^{131}. Most of the adenomatous tissues when prepared as slices behaved normally in all tests used in this study. However, in 5 cases, thiocyanate did not cause discharge of I^{131} from unblocked slices and, in 3 cases, from either the blocked or the unblocked tissue. The reason for this lack of mobilization of tissue I^{131} is not clear. It cannot be due to complete binding of I^{131}, since the TPI of the unblocked slice was not different from that of "normally

behaving" adenomas. Also, the 3 cases in which no mobilization occurred in either blocked or unblocked slices bear proof that the failure to mobilize the I^{131} was not due to biosynthesis of I^{131} into hormone. If it be presumed that some of the I^{131} not ejected from the slice after thiocyanate is in the form of iodide, which is considered to be the form readily mobilized by thiocyanate, the possibility would be present that the iodide in these 8 cases must be somewhat differently held or bound than in normal tissue. Another finding difficult to explain is that some adenomatous tissues were capable of establishing substantial gradients, high uptakes, and a high degree of protein binding, whereas other adenomatous tissues, histologically not different, had low uptakes with high TPI values. This variability may be an expression of a variable state of function of adenomatous tissue during the progression of the disorder. In any case, adenomas with similar microscopic appearance do not metabolize I^{131} in the same way.

It was not possible to obtain simultaneous collateral data on the distribution of labeled compounds formed in the slices. Trunnell and Wade (3), however, noted deviations from normal in the relative proportions of thyroxine and triiodothyronine in hydrolysates of adenomas of 2 patients, but Stanley (4) found that the thyroglobulin of a benign adenoma was not different chemically from that of normal tissue.

The data on carcinomatous tissue are too few for generalization, and in any case do not provide information different from that noted previously from *in vivo* studies. However, thiocyanate did not release I^{131} from slices of those carcinomas which otherwise showed normal I^{131} metabolism. In earlier studies, Pochin and associates (5) demonstrated that thiocyanate did not affect the I^{131} counts over carcinomatous thyroid glands, from which fact one may conclude that protein binding of the tracer I^{131} had occurred. Although a substantial portion of I^{131} in the unblocked slice is not protein-bound, there was no discernible discharging effect of thiocyanate; furthermore, thiocyanate was ineffectual even in the blocked slice, which presumably contained only iodide. The possibility is thus again raised that I^{131} may exist in nondischargeable form in certain disorders of the thyroid.

The findings in cases of Hashimoto's thyroiditis were of particular interest. The tissue in our cases of Hashimoto's thyroiditis was hyperplastic. This was reflected by the high rates of uptake, the high T/M ratios, and the low TPI values. The substantial I^{131} gradients made possible a marked effect of thiocyanate in discharging iodide. Proportionally, more iodide must be present in the tissue in cases of Hashimoto's thyroiditis than in normal tissue. This inference received support from a recent study on Hashimoto's disease by Morgans and Trotter (6). They found that thiocyanate could greatly reduce the counts over the unblocked thyroid one hour after a tracer dose of radioiodide had been administered. A similar

defect may be present in granulomatous thyroiditis, since the iodide^{131} present in thyroid tissue of 2 patients given preoperative tracers amounted to 96 and 71 per cent of the total radioiodine. In another case the TPI of the slice was only 4 per cent, in confirmation of the report of Owen and McConahey (2) that in granulomatous thyroiditis the thyroid cannot convert I^{131} to organic compounds.

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THE EFFECT OF IODIDE ON THE RELEASE OF THYROID HORMONE IN HYPERTHYROIDISM: FURTHER OBSERVATIONS*

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ABSTRACT

This study concerns the effect of iodide ion on the thyroid in 10 thyrotoxic patients. Seventy-two hours after a tracer dose of I^{131}, treatment was started with 1-methyl-2-mercaptoimidazole and continued throughout the study. After seven days, sodium iodide was given orally, followed in some instances by administration of thyrotropin. By means of electrophoretic and chromatographic analyses, it was shown that the method employed allowed observation of the thyroid hormone secretion rate. It would appear that iodide inhibits the secretion rate by neutralizing thyrotropin at some point after pituitary release of thyrotropin. The data suggest that the hyperthyroidism observed in Graves' disease is maintained, if not initiated, by a peripheral concentration of thyrotropin equal to that produced by the daily administration of no more than 6 mg. of thyrotropin daily.

INTRODUCTION

RECENTLY several laboratories have reported investigations dealing with the effect of iodide ion on the rate of release or secretion of thyroid hormone (1–5). All are agreed that the effect is one of slowing this rate. The various mechanisms suggested to account for this effect include, 1) inhibition of pituitary thyrotropin secretion, 2) inactivation of circulating thyrotropin, 3) interference with, or competition with thyrotropin at the level of the thyroid cell, and 4) inactivation of the thyroid cell so as to make it unresponsive to thyrotropin. The present study was planned to prove whether or not the thyroidal I^{131} release curves previously reported actually represented the rate of thyroid "hormone secretion," and to explain further the mechanisms of the iodide effect on this rate.

Received November 25, 1957.

* Presented at the Annual Meeting of the American Goiter Association, New York, N. Y., May 28–30, 1957.

METHOD

The investigation consisted of individual studies conducted on 10 patients with hyperthyroidism: 8 had Graves' disease and 2 had toxic multinodular goiter as defined elsewhere (1). Each patient was hospitalized and studied according to the general plan outlined previously (1). The original tracer dose of I^{131} was 250 microcuries. Seventy-two hours later, oral administration of 1-methyl-2-mercaptoimidazole[1] was started in a dosage of 40 mg. every six hours and continued throughout the remainder of each study. A few studies were performed on patients who received therapeutic doses of I^{131} rather than tracer doses. Iodide was administered orally as sodium iodide, usually after seven days of treatment with MMIA alone; the dose was calculated on the basis of iodide ion. Thyrotropin (Armour) was administered as "thytropar" in certain experiments.

Paper electrophoretic separation of serum proteins was carried out using an E-C Pressure Plate Unit and Whatman #3 paper. The buffer was Spinco B-2 with pH 8.6. The current employed was 2 milliamperes per paper strip at 150 volts and each run was made over a period of twenty hours. Each paper strip was scanned in a Nuclear Company actigraph unit. Unidimensional descending paper chromatographic separation of serum and of urinary iodinated compounds was carried out using Whatman #3 MM paper and a butanol-dioxane-2 molar ammonia (ratio, 4:1:5) solvent system. Each run consisted of an aliquot of the butanol extract of the serum or urine accompanied by samples of standard solutions of iodide, 3-5-diiodothyronine, 3-5,3′-triiodothyronine and tetraiodothyronine. After separation was accomplished, the paper strips were cut widthwise into 1-cm. lengths and each such section was counted separately in a gamma-sensitive Nuclear Company well-type counter for radioactivity. Serum protein-bound iodine determinations were performed according to the method of Zak *et al.* (6).

RESULTS

Radioactivity scanning of the paper electrophoresis analyses showed that serum radioactivity during the period of MMIA administration alone traveled with the beta globulin fraction of the serum proteins. During the administration of both MMIA and iodide simultaneously and MMIA and thyrotropin simultaneously the radioactivity traveled either with the alpha-2 or between the alpha-2 and alpha-1 fractions. The paper chromatographic analyses showed that during the periods of administration of MMIA and MMIA together with iodides, serum radioactivity was located in the region of the tetraiodothyronine spot, with an Rf value of approximately 0.45. During administration of MMIA together with thyrotropin, serum radioactivity was located again in the tetraiodothyronine spot; and also in the region of the iodide spot, with an Rf value of approximately 0.40; and in the region where 3-5-diiodothyronine and 3-5,3-triiodothyronine travel close together, with an Rf value of approximately 0.63. Paper chromatographic analyses performed on urine samples collected during these various periods always revealed the radioactivity in the region of the iodide spot.

[1] Hereafter referred to as MMIA.

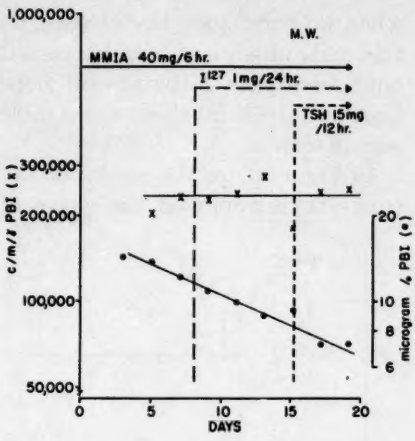

FIG. 1. Patient M.W. The specific activity of the protein-bound fraction of serum iodine during a typical study. The slope values for the thyroid secretion rate in this patient are presented in Figure 6.

The crosses represent the specific activity of serum PBI in terms of counts per minute per microgram, and the dots represent the concentration of serum PBI in micrograms per 100 ml.

MMIA = 1-methyl-2-mercaptoimidazole.

I^{127} = Stable iodide.

Days = Time elapsed after starting MMIA. The radioactive iodine (I^{131}) was given 72 hours prior to day 0.

The vertical scale is logarithmic.

In Figure 1 are shown the results of an investigation of the completeness of the block of thyroid hormone synthesis. The specific activity of the protein-bound fraction of serum iodine, expressed as counts per minute per microgram, rose during the first few days of the study and leveled off before iodide administration was begun. The specific activity then remained constant during the remainder of the study while the serum PBI concentration fell exponentially.

In Figure 2 are seen the results of a study demonstrating the effect of iodide on the curve of radioiodine release from the thyroid, already accelerated by administration of 30 mg. daily of exogenous thyrotropin. Radioactivity (in this and subsequent figures) is expressed as the percentage of the dose remaining in the neck. A dosage of 10 mg. of iodide daily altered the slope of the thyrotropin curve just as quickly and completely as it did

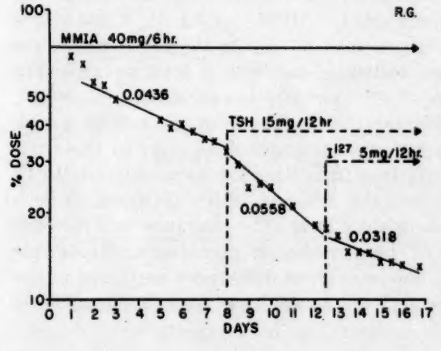

FIG. 2. Patient R.G. Effect of 10 mg. of iodide daily on the TSH-accelerated slope observed during administration of 30 mg. of TSH daily.

In this and subsequent figures:

"% Dose" = Radioactivity remaining in the neck. The statistically calculated value for each slope is noted.

when no exogenous thyrotropin was administered. The slope values (in this and subsequent figures) are calculated by the usual statistical methods.[2] In Figure 2 the second slope was significantly different from the first, and the third slope was significantly different from both the first and second slopes.

In Figure 3 are the results of a study demonstrating the effect of exogenous thyrotropin on the curve of iodide-inhibited thyroidal release of I^{131}. The doses of iodide and thyrotropin were those employed in the study presented in Figure 2. Iodide slowed the release rate as expected and the subsequent administration of thyrotropin did not alter the iodide-inhibited rate. The iodide effect was apparent on the first day of administration of iodide. The second and third slopes were both significantly different from the first, but not from each other.

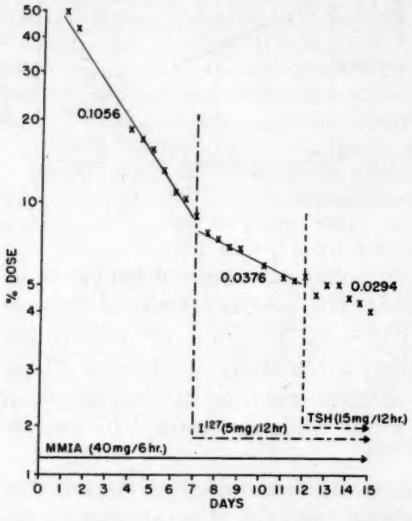

FIG. 3. Patient C.N. Effect of 30 mg. of TSH daily on the decelerated slope observed during administration of 10 mg. of iodide daily.

Shown in Figure 4 are the results of a study identical to that just described in Figure 3, except that the daily dosage of iodide was 5 mg. instead of 10 mg.

In Figure 5 are the results of a study similar to that represented in Figure 3, differing only in that twice the previous daily amount of thyrotropin was administered (60

[2] Methods adapted from "An Introduction to Statistics in the Medical Sciences" by H. C. Batson. Minneapolis, Minn., Burgess Publ. Co., 1956, pp. 54–61. These slopes represent clearance rates, expressed as the fraction of I^{131} in the thyroid removed per day, and were obtained using the standard statistical methods of least squares. The original data, consisting of about 10 values of I^{131} concentration per treatment period, were used to determine the equations describing the movement of the isotope during each treatment situation. Clearance rates and deviations from linearity in the semilogarithmic plane were obtained. Since deviations from linearity were observed to be statistically insignificant in all cases explored, the clearance rates appeared to be a reasonable parameter for comparison of treatment effects. The clearance rate for each treatment period was compared with that of the immediately preceding and following period for the question of significance of difference. These differences are noted in the text. Only those differences in slope value significant at the 1 per cent confidence level were assumed to reflect change.

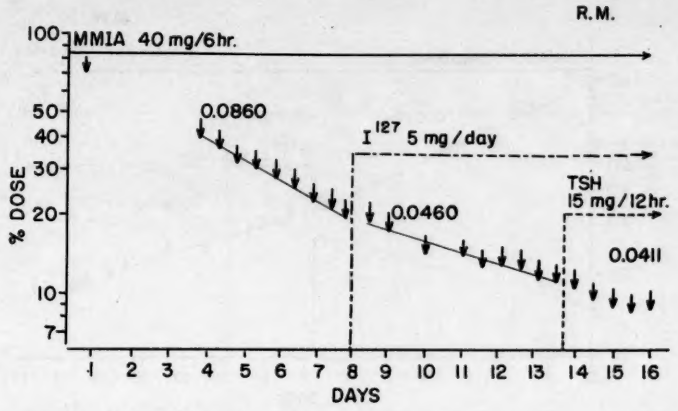

FIG. 4. Patient R.M. Effect of 30 mg. of TSH daily on the decelerated slope observed during administration of 5 mg. of iodide daily.

mg. instead of 30 mg.). Iodide in a dosage of 13 mg. daily again slowed the thyroid release rate. However, this time the administration of thyrotropin significantly changed the iodide-controlled slope, though not back to the value of the pre-iodide slope.

Figure 6 represents the results of a study on the effect of 1 mg. of iodide daily on the thyroidal I^{131} release rate. The rate was significantly slowed, but the slowing effect was not apparent until the fourth day of iodide administration as compared to the interval of one day noted in Figure 3.

In Table 1 the slope values for the study depicted in Figure 6 and for 5 other identical studies are listed. In the first 4 studies the second value is

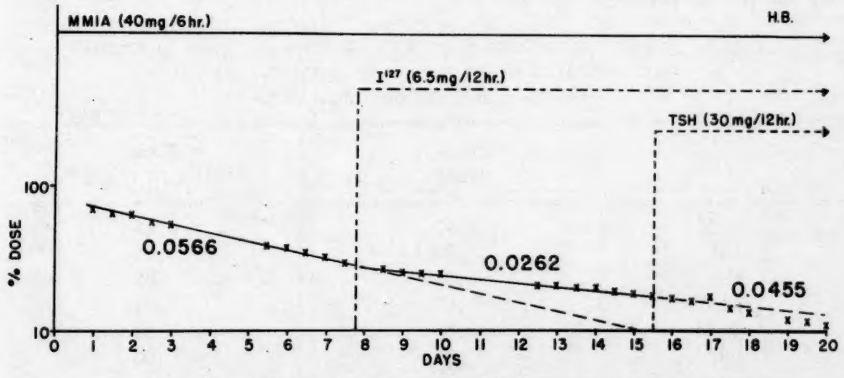

FIG. 5. Patient H.B. Effect of 60 mg. of TSH daily on the decelerated slope observed during administration of 13 mg. of iodide daily.

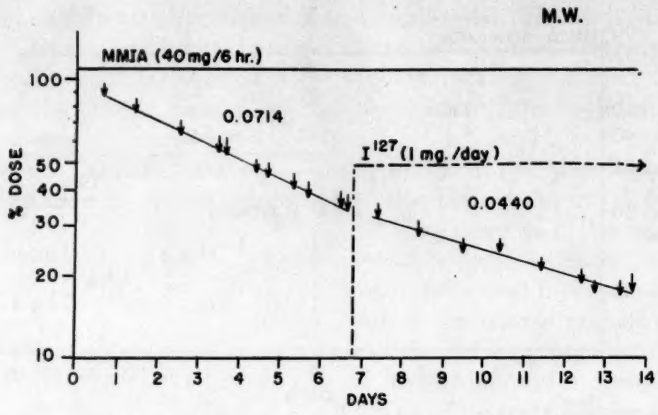

FIG. 6. Patient M.W. Effect of 1 mg. of iodide daily on the MMIA control slope.

significantly less than the first, but in the last 2 there is no difference.

In Figure 7 are shown the results of a study similar to those represented by Figures 3–5. In this instance 1 mg. of iodide and 30 mg. of thyrotropin were given daily. This dosage of iodide inhibited the thyroidal I^{131} release curve. The iodide-influenced release rate was then accelerated promptly by TSH. The addition of an extra 2 mg. of iodide daily did not interfere with the effect of thyrotropin, once started.

In Figure 8 are shown the results of a study similar to that just described. The thyrotropin effect appeared within one day, and when thyrotropin was withdrawn this acceleration disappeared. The reinstitution of thyrotropin in double dosage produced an effect quantitatively similar to that produced by the previous dose.

TABLE 1. SLOPE VALUES* FOR 6 THYROTOXIC SUBJECTS TREATED WITH 40 MG. OF MMIA EVERY SIX HOURS, FOLLOWED BY THE ADDITION OF 1 MG. OF IODIDE DAILY

Patient	Slope, MMIA	Slope, MMIA+1 mg. I^{127}
M.H.	0.07	0.03
J.H.	.02	.01
F.D.	.04	.02
M.W.	.07	.04
W.M.	.05	.04
I.J.	.03	.03

* The values are expressed as a percentage of the dose of I^{131} remaining in the thyroid that is lost each day.

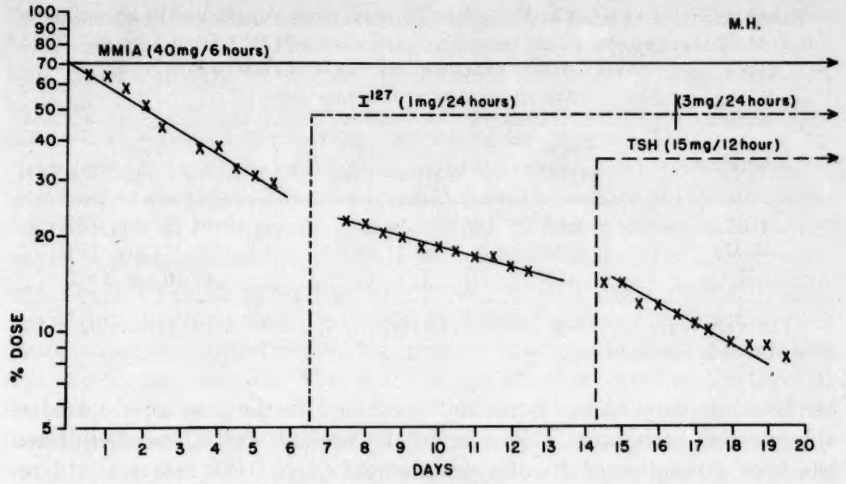

FIG. 7. Patient M.H. Effect of 30 mg. of TSH daily on the decelerated slope observed during administration of 1 mg. and then 3 mg. of iodide daily.

Listed in Table 2 are the slope values obtained in 3 studies similar to that noted in Figure 8. In each instance iodide produced a significant slowing of the release rate. In Patient M. W., thyrotropin in the usual dosage seemed to increase the thyroidal I^{131} release rate but this was not statistically significant. In Patients W. M. and J. H. smaller amounts of thyrotropin still significantly increased the release rate.

DISCUSSION

At least two ideas are implicit in the statements made in previous publications concerning thyroid "hormone secretion" or "hormone release" rates. First, the radioactivity released from the thyroid in these studies

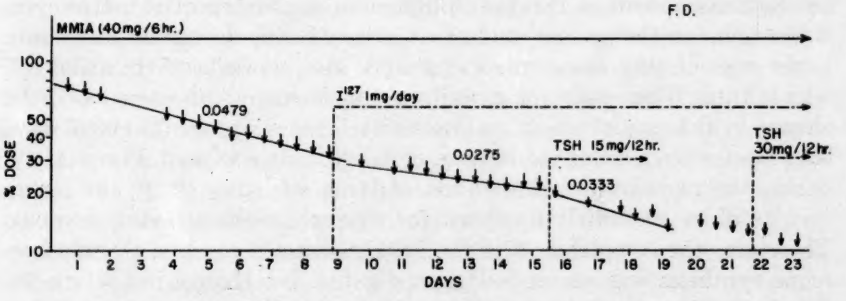

FIG. 8. Patient F.D. Comparison of the effects of 30 mg. and 60 mg. of TSH daily on the decelerated slope observed during administration of 1 mg. of iodide daily.

TABLE 2. SLOPE VALUES* FOR 3 THYROTOXIC SUBJECTS TREATED WITH 40 MG. OF MMIA EVERY SIX HOURS (CONTROL); THEN WITH MMIA PLUS 1 MG. OF IODIDE DAILY, FOLLOWED BY THE ADDITION OF THYROTROPIN IN VARIOUS DOSAGES

Patient	Slope, MMIA	Slope, MMIA+1 mg. I^{127}	Slope, MMIA+1 mg. I^{127}+TSH
M.W.	0.07	0.04	0.04 (30 mg. TSH)
W.M.	.05	.04	.06 (15 mg. TSH)
J.H.	.02	.01	.02 (6 mg. TSH)

* The values are expressed as the percentage of the dose of I^{131} remaining in the thyroid that is lost each day.

has been assumed to be "hormonal" activity, on the basis of a considerable amount of indirect evidence. In the present study this assumption has been strengthened by the demonstration that the radioactivity released into the blood during these studies acted like hormonally-bound radioactivity, *i.e.*, it traveled with the same serum proteins as does tetraiodothyronine and it moved along a paper chromatogram with Rf values that identified it as bound to 3-5,3′-triiodothyronine and tetraiodothyronine. Second, the curves representing these data have been assumed to be "secretion" curves. As noted elsewhere, a small "leak" in the MMIA-induced block of thyroid hormone synthesis can permit dilution of the thyroid's labeled hormone pool with newly formed, unlabeled hormone when relatively large amounts of stable iodides are administered (1, 7). Such dilution would effect a decrease in the specific activity of the thyroid hormone pool. As a result, a "leak" would make possible a decrease in the rate of loss of radioactivity from the thyroid area even though the rate of discharge or secretion of hormone from the thyroid remained static or in fact increased. The use of relatively large doses of iodides precluded an accurate assessment of this possibility in an earlier report (1). However, it was found in the present study that a daily iodide dosage that was only 1 per cent of the dosage used originally also slowed the thyroidal I^{131} release rate. When only 1 mg. daily of iodide was administered (1), the change in the rate of loss of radioactivity became apparent several days later than when a dosage of 100 mg. of iodide daily was used. Fortunately, during the period of administration of 1 mg. of iodide daily, the serum could still be accurately analyzed for its protein-bound iodine content. Therefore, the completeness of the MMIA-induced block of thyroid hormone synthesis was successfully investigated. No change in the specific activity of the protein-bound fraction of the serum iodine occurred during

the interval when the rate of loss of radioactivity from the thyroid was being slowed by iodide. There was no apparent entry of unlabeled hormone into the labeled thyroid hormone pool during this interval. The conclusion is that the curves being studied in the present, and similar, investigations can indeed be called the secretion curves of the thyroid gland.

The rest of the results of the present study concern the mechanism of the inhibition of the thyroid hormone secretion rate by iodide. If this inhibition were a result of interference by iodide with pituitary secretion of thyrotropin, the administration of iodide to a subject whose thyroid secretion rate was already under significant stimulation by exogenous thyrotropin should not alter that secretion rate. Yet iodide did alter the secretion rate under these circumstances in the present study, and in a similar one reported by Solomon (5). Also, if the iodide effect occurred at the level of the pituitary, a reversal of the foregoing order of administration of thyrotropin and iodide should show that the dose of exogenous thryotropin which accelerates the thyroid hormone secretion rate when given without any simultaneous iodide should still accelerate the secretion rate already slowed by prior iodide administration (8). This was not the case. Therefore, the reasonable conclusion is that iodide does not inhibit the rate of thyroid hormone secretion, at least in thyrotoxic man, by inhibiting pituitary thyrotropin secretion. Another possibility investigated was that iodide renders the thyroid entirely unresponsive to thyrotropin, as would happen if the enzyme or enzymes controlling proteolysis of thyroglobulin were completely inhibited in a noncompetitive manner by the minimally effective dose of iodide. If this were the mechanism of the iodide effect, no amount of exogenous thyrotropin should accelerate that thyroid hormone secretion rate already significantly slowed by iodide. This is not the case, since under the conditions employed in this study the thyroid was responsive to some dose of thyrotropin, albeit a relatively large one. This leaves as the best of the originally discussed explanations the one which postulates an alteration of circulating thyrotropin by iodide ion or one which postulates a competition between thyrotropin and iodides, at the level of the thyroid cell, for control of the proteolytic enzymes governing hormone release. There are some data in the literature supporting the suggestion that the iodide effect in thyrotoxic man is the result of inactivation of thyrotropin by means of a loose combination of the two compounds, occurring peripherally. The "in vitro" work of Albert and associates showed that reducing agents, such as the thioureas, could reverse the inactivation of thyrotropin that was caused by prior incubation with iodine (9). Also the work of Postel showed that by means of zone electrophoresis active thyrotropin and active thyroxine could be separated from a solution of thyrotropin

inactivated by prior incubation with thyroxine (10). Apparently the alterations of thyrotropin so produced do not involve any basic structural changes.

Although various doses of iodide have been used, 1 mg. administered daily appeared to be close to the smallest amount that will produce a significant response in thyrotoxic man. Evidence for this is contributed by the fact that in patients receiving this dose, the iodide-period slope value was significantly less than the preceding slope value in only 4 of 6 patients with Graves' disease. Moreover, this difference, when present, did not become apparent as quickly as was noted when larger doses of iodide were employed (1). This minimal dose range is in agreement with the results of other studies planned to estimate the minimal effective dose of iodide ion in Graves' disease (11).

The final conclusions concern the dose relationship between iodide ion and thyrotropin. During the minimal inhibitory dosage of 1 mg. of iodide daily, the daily administration of an amount of thyrotropin which later did not alter the secretion rate slowed by 5 mg. of iodide daily, did rapidly and significantly accelerate the secretion rate. However, the effect was not quantitatively similar to that seen when thyrotropin alone was administered to a thyrotoxic subject receiving MMIA (8). Since various doses of thyrotropin seemed to produce the same effects when administered with a dosage of 1 mg. of iodide daily, it was assumed that the thyrotropin was being given in excess. Further study showed that the minimal dosage of thyrotropin that will alter the secretion rate under this degree of iodide inhibition is approximately 6 mg. daily. This dose seems to be close to the minimal effective dose of thyrotropin, since in the present study the acceleration became apparent only after several days of thyrotropin administration instead of after twelve hours, as noted in other studies when there was no simultaneous administration of iodide (8).

On the basis of these results, an estimate of the amount of thyrotropin necessary for the maintenance of Graves' disease can be made. Because 1 mg. of iodide daily appears to be close to the minimal effective dose of iodide, and because 6 mg. of thyrotropin daily just reverses this inhibition, it is likely that in Graves' disease there is a blood concentration of effective, un-neutralized circulating thyrotropin that is less than the concentration produced by the daily intramuscular administration of 6 mg. of exogenous thyrotropin.

In Figures 9 and 10 are the composite statistical results of the data presented in earlier figures. The scatter data in Figure 9 suggest that the clearance rate of the MMIA-plus-iodide period (K_2) is dependent on the rate which is effective during the MMIA period (K_1). That is, the iodide effect is not the sole determinant of K_2; rather, K_2 is in part a function of the

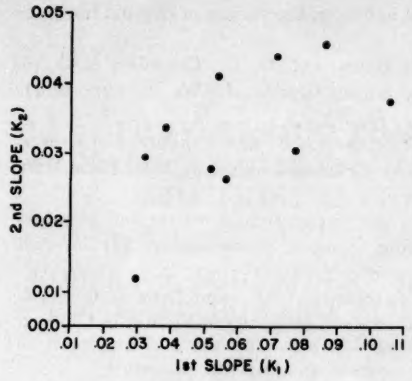

FIG. 9. Relationship of iodide and control slopes in each of the 10 studies.
1st slope = MMIA slope (K_1).
2nd slope = MMIA + I^{127} slope (K_2).

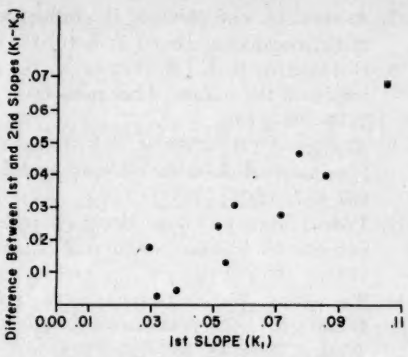

FIG. 10. Relationship of the slope differences ($K_1 - K_2$) to the control slope in each of the 10 studies.

biologic peculiarities of the patient and the disease, as represented by K_1. The scatter data in Figure 10 suggest that the iodide effect (K_1–K_2) is directly dependent on the thyroid hormone secretion rate (K_1) and is not a constant factor. Furthermore, on close inspection of the data in Figures 9 and 10 there appear to be 2 distinct curves lying parallel to one another. Whether these curves actually represent 2 distinct populations cannot be answered by the data. The cases have been analyzed as regards age and sex of the patient, type of disease, severity of the clinical situation, size of the goiter, dose of iodides, type of counting geometry and equipment, and original counting rates but no explanation for such a population split has been found.

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OLFACTORY PERCEPTION THRESHOLDS IN HYPOGONADAL WOMEN: CHANGES ACCOMPANYING ADMINISTRATION OF ANDROGEN AND ESTROGEN*†

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ABSTRACT

Olfactory perception thresholds and nasal mucous membrane function were measured over prolonged periods in 3 hypogonadal females; in 1 of these subjects nitrogen and sodium balance were also studied. Observations were made during periods of estrogen therapy (equilin sulfate, Premarin, estradiol-17α,3-monosulfate), of androgen therapy (testosterone propionate and nortestosterone), and of placebo administration. Estrogenic and androgenic effects were ascertained by measuring urinary gonadotropin and 17-ketosteroid excretions, respectively. Olfactory acuity during periods of placebo administration was not significantly different from acuity measured over long periods in 6 healthy women with normal gonadal function, but in the hypogonadal group there was a tendency toward pallor and swelling of the nasal membranes. Olfactory acuity increased significantly in 2 hypogonadal subjects while they were receiving estrogens, but this was not associated with significant changes in nasal function; throughout the study, their olfactory threshold values and corresponding urinary gonadotropin levels were correlated positively. In contrast, olfactory acuity decreased significantly in the third hypogonadal subject while she was receiving androgens, and there was a positive correlation between acuity and 17-ketosteroid excretion. During androgen therapy, this subject showed a modest increase in nasal swelling and secretions which might have contributed to the observed decrease in olfactory acuity. There was a positive correlation between nitrogen excretion and olfactory acuity, but there was no such correlation between sodium excretion and acuity. The mechanisms by which estrogens improve and androgens depress olfactory acuity in hypogonadal women remain obscure. However, there is a suggestion that local changes in the nasal membranes may contribute to these differences.

Received August 12, 1957.

* Presented at the Annual Meeting of the Southern Society for Clinical Research, New Orleans, La., January 26, 1957.

† These studies were supported in part by research grant B-286 from the section on Neurological Diseases and Blindness, National Institutes of Health, and by a grant from Ayerst Laboratories, New York City.

IT HAS been observed that women surpass men in olfactory acuity (1). Recent quantitative studies from this laboratory not only confirmed the lower threshold for smell among women (2), but indicated that olfactory acuity is decreased during menstruation (2). Elsberg and associates (3) reported that acuity is increased during the menstrual period. Despite these divergent findings, the fact that the level of perception varies during a phase in the menstrual cycle suggests that olfactory acuity may be influenced by gonadal hormones. The present study is a report of measurements of olfactory perception made over long periods in 3 hypogonadal women who were receiving, intermittently, androgens or estrogens for therapeutic reasons. Concomitantly, metabolic data were secured and observations were made of the physiologic status of the nasal mucosa.

METHODS

1. *Determination of olfactory perception threshold*

Citral[1] was used as the odorant throughout the study. Perception thresholds were determined in a test room (olfactorium) controlled for temperature and humidity, where various accurately measured amounts of the odorant were presented. Threshold was defined as the smallest amount of odor which the subject could identify on each occasion. A description of the olfactorium and of the detailed steps of measurement is in a previous publication (2).

2. *Observations on the nasal mucosa*

Immediately after the threshold was determined, observations were made on the state of the subject's nasal mucosa and the presence or absence of nasal obstruction. Through an illuminated nasal speculum, estimates were made of the degree of mucosal swelling, the amount of secretions, the degree of obstruction (scale, 0 to 4+), and the color of the nasal mucous membrane (using a color scale). These arbitrary scales are described elsewhere (4).

3. *Urinary excretion of gonadotropin*

Urinary gonadotropin assays were carried out according to the method of Klinefelter, Albright and Griswold (5). Cellophane dialysis was not used. Values were expressed in terms of 48, 96, 192 and 384 mouse units per twenty-four hours. Normal values are less than 96 m.u. per twenty-four hours. Each value was determined for the urine collected twenty-four hours prior to the measurement of the olfactory threshold.

4. *Urinary excretion of 17-ketosteroids*

Urinary 17-ketosteroid values were determined utilizing the Zimmermann reaction and following the technique of Klendshoj, Feldstein and Sprague (6). Results greater than 14 mg. or less than 6 mg. per twenty-four hours are abnormal for adult women.

5. *Nitrogen and sodium balance*

In the single subject studied on a metabolic ward, nitrogen balance (Gm./24 hours)

[1] Citral, an aromatic organic compound with a citrus-like odor, was kindly furnished by Fritzsche Brothers Co., New York, N. Y.

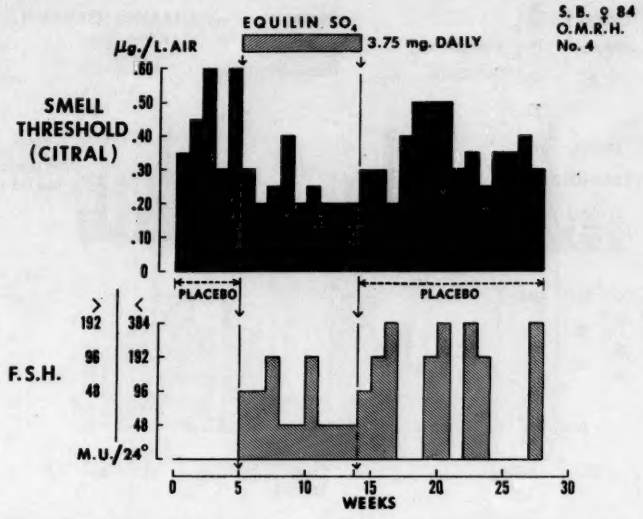

FIG. 1. Week-to-week variations in olfactory thresholds (solid bars) and corresponding gonadotropin (F.S.H.) excretion levels (cross-hatched bars) in a postmenopausal woman with osteoporosis (S.B.). Note the lower olfactory thresholds (increased olfactory acuity) during a period of equilin sulfate therapy.

and sodium balance (mEq./24 hours) were estimated from the difference between the intakes and the urinary and fecal excretions.

SUBJECTS AND PROCEDURES

1. *Control female subjects*

Olfactory threshold measurements and nasal mucous membrane observations were made on 6 healthy women with apparently normal gonadal function who were not receiving hormones. Their ages ranged from 15 to 40 years. Observations made during upper respiratory infections and during menstrual periods were excluded.

2. *Hypogonadal female subjects*

Detailed observations were made on 3 hypogonadal women: 1) S.B., an 84-year-old woman with osteoporosis who received estrogens periodically for the complaint of backache; 2) M.M., a 31-year-old woman, surgically castrated, who received estrogens intermittently; and 3) L.R., a 69-year-old woman who at times was receiving androgens for complaints referable to osteoporosis.

Subjects S.B. and M.M. were followed as out-patients over periods of twenty-eight to forty-three weeks, respectively. Observations were made weekly. The study of these 2 patients was initiated to determine the effects of estrogens on urinary gonadotropin excretion (7). At each visit the patient was seen between 9:00 and 10:00 A.M. having eaten nothing since the prior evening meal; she brought in the previous 24-hour urine (7:00 A.M. to 7:00 A.M.) for assay of gonadotropin. Each patient was followed clinically by one of the authors (R.P.H.), who prescribed hormones or placebos and made clinical

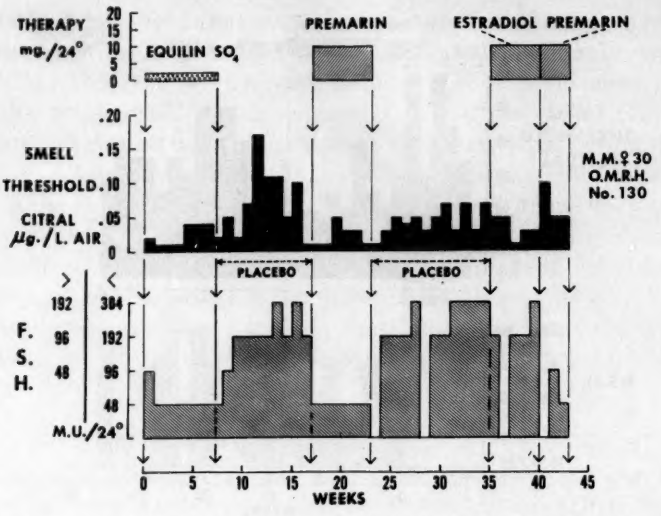

FIG. 2. Week-to-week variations in olfactory thresholds (solid bars) and corresponding gonadotropin (F.S.H.) excretion levels (cross-hatched bars) in a young hypogonadal subject (M.M.). Note the lower olfactory thresholds (increased olfactory acuity) during periods of estrogen therapy. Estradiol = estradiol-17α,3-monosulfate.

observations. All estrogens and placebos were given orally. Subject S.B. received equilin sulfate, 3.75 mg. daily, for eight seeks; both before and after this therapy she received placebo (Fig. 1). Subject M.M. received 2.5 mg. of equilin sulfate daily for seven weeks, 10 mg. of Premarin[2] daily for six weeks (and later for three weeks after estradiol); and 10 mg. of estradiol-17α,3-monosulfate daily for five weeks; placebos were administered in the intervening periods (Fig. 2). At each clinic visit a trained technician determined the olfactory threshold and examined the nasal mucosa, without knowledge of the patient's clinical course or the treatment program.

Subject L.R. was followed for eighty-four days as an in-patient on a metabolic ward, where intake was controlled. The study of this patient was initiated to determine the effects of androgen administration on serum lipids and lipoproteins (8). The urinary and fecal outputs were assayed for sodium and nitrogen, and the 17-ketosteroids were measured daily. Olfactory threshold was determined at 4:00 P.M. at varying intervals —sometimes on four or five successive days and otherwise once every fourth or fifth day. The subject received a single intramuscular injection of 600 mg. of testosterone propionate in oil on the thirteenth day of the study, and daily oral nortestosterone (50 mg. for twelve days, 100 mg. for ten days and 200 mg. for six days) from the forty-eighth to the seventy-sixth day of the experiment (Fig. 3). Throughout the period of study she received bedtime sedation, vitamins and tincture of belladonna but did not receive placebos, as such, when not taking hormones.

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[2] Mixed conjugated estrogens in the sulfate form—Ayerst Laboratories, New York City.

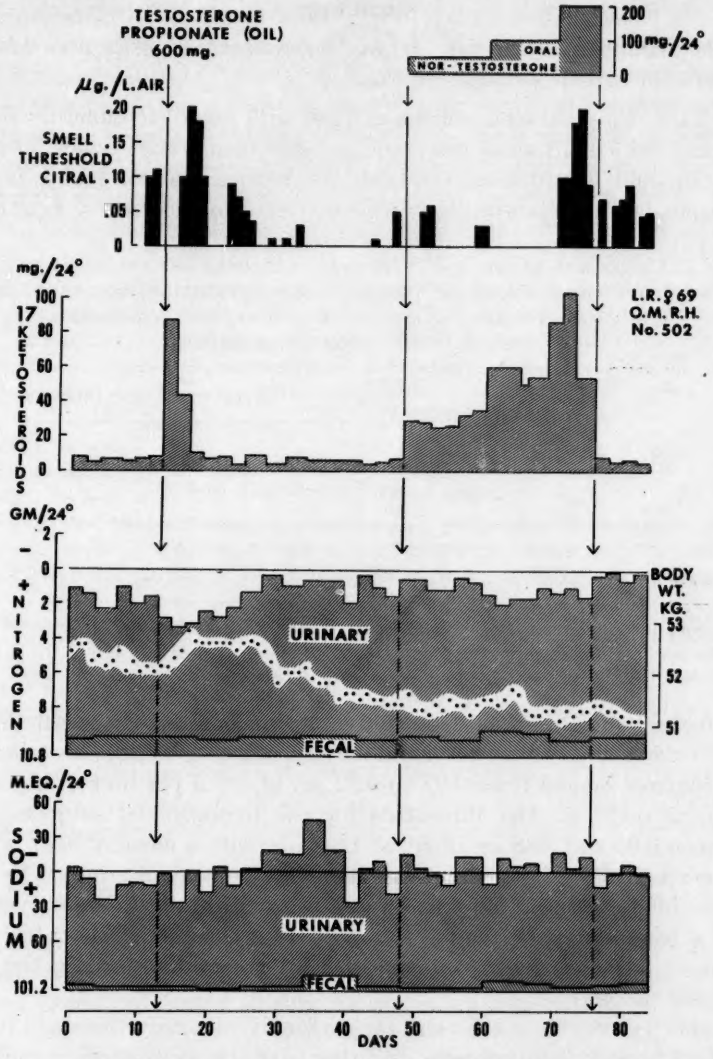

FIG. 3. Comparison of day-to-day variations in olfactory threshold, 17-ketosteroid excretion, and nitrogen and sodium balances in a hospitalized postmenopausal subject (L.R.) who received androgen therapy intermittently. Higher olfactory thresholds (decreased olfactory acuity) during androgen therapy were associated with greater 17-ketosteroid excretion and increased nitrogen retention, but not with significant changes in sodium balance. Nitrogen intake was constant at 10.8 Gm./24 hours. Fecal and urinary N_2 output is plotted upward from the intake line. Positive balance is shown as a clear area under the zero line and negative balance as a hatched area above the zero line. Sodium balance is charted in the same manner as nitrogen balance.

RESULTS

Olfactory acuity and nasal function in 3 hypogonadal women versus 6 healthy women with normal gonadal function

In Table 1 is a summary of the findings with regard to olfactory thresholds and the condition of the nasal mucous membranes (color, swelling, secretion and obstruction) for both the normal control group and the hypogonadal subjects when they were not receiving hormones. The thresh-

TABLE 1. MEANS AND RANGES FOR OLFACTORY THRESHOLD AND FOR NASAL MEMBRANE FUNCTION (COLOR, SWELLING, SECRETION AND OBSTRUCTION) IN A GROUP OF 6 HEALTHY FEMALES AND IN A GROUP OF 3 HYPOGONADAL FEMALES WHO WERE NOT RECEIVING HORMONES

		Threshold for citral (μg./L. air)	Nasal membrane function			
			Color (0–100)	Swelling (0–4+)	Secretions (0–4+)	Obstruction (0–4+)
Normal females [6]	Mean	0.12	54	1.5	1.5	1.5
	Range	0.02–0.32	50–60	1.5–2.0	1.5–2.0	1.0–1.5
Hypogonadal females [3]	Mean	0.16	48	1.8	1.5	1.1
	Range	0.05–0.38	42–55	1.5–2.0	1.0–2.0	0.75–1.0

old mean values are based on individual means and the nasal function means are derived from individual median values. Olfactory thresholds for the controls ranged from 0.02 to 0.32 μg. of citral per liter of air, with a mean of 0.12 μg. The thresholds for the hypogonadal subjects varied between 0.05 and 0.38 μg. of citral/L. of air, with a mean of 0.16, which is not statistically[3] higher than the mean for the control group. The group means for the 4 nasal functions were not significantly different, yet there was a trend toward paleness (mean of 48 compared to a control group mean of 54) and swelling (mean of 1.8 compared to 1.5) in the hypogonadal subjects.

Table 2 shows the means and ranges for the olfactory threshold for each of the 3 hypogonadal subjects, together with the corresponding individual median values for the 4 nasal functions during both placebo and hormone periods. It may be seen that the threshold fell significantly[3] in the 2 subjects receiving estrogen (from 0.38 to 0.24 μg. of citral/L. of air for Subject S.B.—$P < .01$; and from 0.06 to 0.03 μg. for Subject M.M.—$P < .05$), but

[3] The *t*-test was used to determine the significance of the differences between the means.

rose significantly ($P < .05$) in Subject L.R. during periods when she was receiving androgen. In Subject S.B. the median nasal membrane color decreased significantly[4] ($P < .02$) from 42 to 35 while she was receiving estrogens. Otherwise there were no statistically significant differences in nasal function ascribable to the hormones in these subjects. However, there was a trend toward increase in secretions and obstruction in Subject L.R. during androgen therapy; though seemingly slight, it might be relevant to the associated elevated olfactory threshold. Furthermore, there

TABLE 2. MEANS AND RANGES FOR OLFACTORY THRESHOLD, AND MEDIANS AND RANGES FOR NASAL MEMBRANE FUNCTION IN 3 HYPOGONADAL SUBJECTS WHEN THEY WERE NOT RECEIVING HORMONES AND WHEN THEY WERE RECEIVING EITHER ESTROGEN OR ANDROGEN

Subject	Treatment	Threshold for citral (μg./L. air)		Nasal membrane function							
				Color (0–100)		Swelling (0–4+)		Secretions (0–4+)		Obstruction (0–4+)	
		Mean	Range	Median	Range	Median	Range	Median	Range	Median	Range
S.B.	No hormone	0.38	0.20–0.60	42	32–55	2.0	0.5–2.5	2.0	0.5–3.5	1.0	0.0–2.5
	Estrogen	0.24*	0.20–0.40	35**	25–47	1.0	1.0–2.5	1.0	1.0–1.5	1.0	0.5–1.5
M.M.	No hormone	0.03	0.01–0.17	47	42–60	2.0	1.0–3.5	1.5	0.5–3.5	1.5	0.0–4.0
	Estrogen	0.03†	0.01–0.10	47	37–57	1.5	0.5–3.0	1.0	0.0–4.0	1.5	0.0–3.5
L.R.	No hormone	0.05	0.01–0.11	55	42–60	1.5	0.0–2.5	1.0	1.0–2.0	0.75	0.0–1.5
	Androgen	0.10†	0.03–0.20	51	40–62	0.75	0.0–2.0	1.5	0.5–2.0	1.0	0.0–2.0

* = $P < .01$.
** = $P < .02$.
† = $P < .05$.

was a tendency for a decrease in secretions during estrogen therapy in the 2 subjects who received these hormones. This response is opposite to the increased secretions noted during androgen administration in Subject L.R.

Increase in olfactory acuity during estrogen therapy in an 84-year-old woman with osteoporosis

Figure 1 shows the week-to-week variations in olfactory acuity and in urinary gonadotropin values for the 28-week period of observation. When

[4] The non-parametric median test (9) was used to determine the significance of differences between the medians.

Patient S.B. was not receiving hormones but was receiving placebos her olfactory thresholds for citral varied from 0.20 to 0.60 μg./L. of air, with a mean value of 0.38 μg. For the same periods the urinary gonadotropin, when measured, showed the expected high values of the hypogonadal subject. Her symptoms, including backache, were minimal during this time. Then for a nine-week period she received equilin sulfate daily, during which time her olfactory threshold for citral decreased significantly ($P < .01$) to a mean value of 0.24 μg./L. of air, with a range from 0.20 to 0.40 μg. The corresponding gonadotropin values were lower and indicated a good effect obtained by estrogen therapy, when compared to higher values during subsequent placebo administration. Minimal symptoms were unaltered during estrogen therapy.

Increase in olfactory acuity during estrogen therapy in a 30-year-old woman with menopausal symptoms after oophorectomy

Figure 2 shows the week-to-week olfactory threshold levels and the corresponding urinary gonadotropin values over a 43-week observation period in Patient M.M. For the twenty-one weeks when the patient was not receiving estrogens, the olfactory thresholds for citral ranged from 0.01 to 0.17 μg./L. of air, with a mean value of 0.06 μg. Except for the first two values, the corresponding gonadotropin titers exceeded 96 m.u., indicating that the patient was, indeed, hypogonadal. Throughout these control periods, no active estrogens were administered and hot flashes were experienced within ten days of stopping the estrogen. For four periods during the study the subject received estrogens as indicated in Figure 2. Within ten days of starting estrogen therapy, hot flashes were relieved. During these estrogen periods the olfactory thresholds were significantly lower than during the placebo periods, with values ranging from 0.01 to 0.10 μg. of citral per liter of air, with a mean value of 0.05 μg. ($P < .05$). The accompanying gonadotropin titers were <96 m.u. per twenty-four hours (indicating good estrogenic effect), except while the patient was receiving the relatively weak estrogen, estradiol-17α,3-monosulfate; during the latter period, gonadotropin ranged from >96 to >192 m.u. per day. When olfactory threshold values throughout the study were studied in relation to the corresponding gonadotropin values, a significant positive correlation[5] was found ($P < .01$).

Decrease in olfactory acuity during androgen therapy in a 69-year-old woman with osteoporosis

Figure 3 shows olfactory threshold values, corresponding 17-ketosteroid excretion levels, and nitrogen and sodium balances for an 84-day period of

[5] Spearman rank correlation coefficient (9).

observation while Subject L.R. was in a metabolic ward. On the thirteenth day, the patient received a single injection of 600 mg. of testosterone propionate in oil, then no hormones till the forty-eighth day, when she began a 28-day period of oral nortestosterone. During the 3 control periods, when no hormones were given but the patient received her customary night-time sedation, vitamins and tincture of belladonna, she complained of backache and other nonspecific symptoms. Her olfactory threshold for citral ranged from 0.01 to 0.11 μg./L. of air, with a mean value of 0.05 μg. The urinary 17-ketosteroid levels were generally low, averaging 8 mg. daily. During the periods when she received androgens, including the twelve days after the single injection of testosterone propionate, the olfactory threshold was significantly higher ($P < .05$), ranging from 0.03 to 0.20 μg./L. of air, with a mean value of 0.10 μg. The associated 17-ketosteroid values were elevated, averaging 38 mg. per day. The patient was relatively asymptomatic during androgen therapy. When all values were considered, a significant positive correlation was found ($P < .01$) between 17-ketosteroid excretion and olfactory threshold.

It may be noted in Figure 3 that throughout the observation period there was a positive nitrogen balance, but that in association with androgen administration the balance was relatively more positive, as might be expected. The correlation was high ($P < .01$) between nitrogen excretion values (constant intake) and the corresponding olfactory threshold values. The patient gained some weight after the single injection of testosterone propionate but failed to do so later when receiving oral nortestosterone.

Sodium balance (Fig. 3) was little affected by androgen therapy in this subject, except for a ten-day period of negative balance occurring twelve to fifteen days after the single injection of testosterone propionate. There was no correlation between sodium balance and olfactory threshold.

DISCUSSION

In the hypogonadal state (when not receiving hormones) only 1 (S.B.) of the 3 subjects had an abnormally elevated olfactory threshold compared to the healthy controls. This subject's poor olfactory acuity under these circumstances could possibly be explained as due to old-age degeneration of the olfactory end-organ, such as might be expected in regard to vision or hearing. The nasal membrane showed some pallor and swelling, increased secretions, and obstruction compared to that of the healthy controls. These variations were not statistically significant, however. Such changes could contribute to poor olfactory acuity, since the odorant would to an extent be impeded in reaching the olfactory membrane. There was a significant decrease in olfactory threshold when the patient was receiving estrogens. This was a *relative* improvement in acuity (the threshold level

reached would be considered high for a healthy female subject) and was accompanied by a modest decrease in swelling, secretions and obstruction and an increase in pallor. Whether or not these alterations in the membrane could account for the improvement in acuity remains speculative, since the changes were not marked.

The other 2 hypogonadal subjects, when not receiving hormones, had normal olfactory thresholds (as in the controls). M.M., the 30-year-old woman who was a surgical castrate, had a normal threshold when not receiving hormones and her nasal membranes differed from those of the healthy controls only in their wider range of variability and a tendency at times towards increased swelling, secretions and obstruction. Her olfactory threshold fell significantly during estrogen therapy, and this was accompanied by a slight decrease in swelling, secretions and obstruction. The impression remains that the increased olfactory acuity which accompanies estrogen therapy is possibly due to local changes in the nasal membrane in the direction of decreased swelling, obstruction and secretions.

The 70-year-old subject with osteoporosis (L.R.) had a normal threshold when not receiving hormones; at these times her nasal membranes were similar to those seen in the healthy controls. When receiving androgens, there was a significant decrease in olfactory acuity, which was accompanied by a slightly more moist and more obstructed nose—the opposite of the changes seen in hypogonadal subjects receiving estrogens. It is conceivable that the effect of hormones on olfactory acuity depends on their ability to alter nasal membranes locally, and the effects observed depend in part on the status of the nasal membranes at the time therapy begins. It would be of great interest to see 1) what effect androgens would have had on Subjects S.B. and M.M., who received estrogen, and 2) the effect of estrogen in Subject L.R. Certainly the status of the nasal membranes has a bearing on olfactory acuity. The relationship is commonly observed in the case of the common cold, in which obstruction, swelling and increased secretions are accompanied by poor olfactory acuity. The present study involves observations on the nasal membranes which may be pertinent to the transport of the odorant to the end-organ. However, no observations were made of the local environment of the olfactory membrane itself, and such changes might be particularly pertinent.

Olfactory acuity is poor or nil in patients with atrophic rhinitis, a condition in which atrophy of the nasal membrane, dryness and redness are typical features. Local application of estrogens is said to improve atrophic rhinitis (10), and with regeneration there is a return toward normal olfactory acuity. Observations have also been reported which show that changes in the genital tract in hypogonadal women (atrophy, dryness, redness and poor turgor) are accompanied by similar changes in the nasal mucous

membranes (10). However, observations of the genital tract were not made in our series. It would seem unlikely that atrophy or regeneration of the nasal membrane due to age or to systemic hormone administration would explain the alterations which were observed, particularly since these changes occurred so rapidly.

It would also seem unlikely that the increases and decreases in threshold were related to the patient's symptoms or relief of symptoms. Both estrogens and androgens gave relief, but administration of the former was accompanied by a decrease in olfactory threshold and the latter by an increase in threshold.

In Subject L.R., nitrogen retention due to androgen therapy was associated with poor olfactory acuity. Whether such changes in nitrogen balance could account for local changes in the olfactory membrane is purely speculative at this time. Changes in electrolyte levels attributable to estrogens or androgens could conceivably alter the production or quality of nasal mucus and the swelling of the membrane, but there was no evidence of this in the present study.

One could speculate on the possible central nervous system effects of these hormones but such changes in neural function in association with the administration of, or deficiency in, hormones have not been demonstrated with regard to the special senses and were not studied here. However, there is suggestive evidence that hormones do alter the sensitivity or excitability of nerve cells through alteration in the synthesis or metabolism of acetylcholine or cholinesterase (11). Torda and Wolff (12) found that androgen, progesterone or adrenocortical hormone depressed the synthesis of acetylcholine, whereas estrogen and pregneninolone increased it.

The present study should be continued and extended to more subjects. It would be of interest, for example, to study the effects of these hormones on women with normal gonadal function and on men who are gonadally deficient or who have normal gonadal function but require androgens or estrogens for therapeutic reasons.

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URINARY CORTICOSTEROID EXCRETION PATTERNS IN PATIENTS WITH ADRENOCORTICAL DYSFUNCTION*

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ABSTRACT

A technique is described for obtaining patterns of the urinary excretion of steroids containing Δ^4-3-keto- and α-ketolic groupings. The patterns in normal subjects showed the presence of pregnane-3α,11β,17α,21-tetrol-20-one (THF), pregnane-3α,17α,21-triol-11,20-dione (THE), cortisol (compound F), cortisone (compound E), and 5 unidentified compounds X_1, X_6, X_9, X_{10} and X_{11}. In addition, corticosterone (compound B) was excreted in 1 patient, and pregnane-3α-11β,21-triol-20-one (THB) in 2 normal subjects. Compounds THE and THF were the chief metabolites.

In 3 patients with Cushing's syndrome similar compounds were present on the paper chromatograms. However, they were excreted in much larger amounts. During administration of ACTH, all of the α-ketolic steroids were further increased, the greatest rise occurring in the active hormones F and E. There was no increase in the amounts of the fast-moving compounds X_9 to X_{11}. After adrenalectomy these substances disappeared from the patterns, and compounds X_1, THF, THE, F, E and X_6 were observed as metabolites of the administered cortisone.

A patient with panhypopituitarism presented qualitatively a normal distribution of corticosteroids. Quantitatively, however, the amounts of all the individual compounds were decreased and remained unchanged after administration of ACTH. A patient with Addison's disease showed no α-ketolic steroids, except for traces of THF and THE, in both the untreated state and during administration of ACTH.

A boy with congenital adrenal hyperplasia had an abnormal excretion pattern. He excreted large amounts of pregnane-3α,17α,21-triol-20-one (THS) and smaller amounts of 11-desoxycortisol (compound S), both of which became almost completely suppressed by cortisone therapy. A 21-year-old girl with the adrenogenital syndrome had a normal pattern of corticosteroid excretion. After the administration of ACTH, relatively large amounts of compound THS could be demonstrated in the urine. Therapy with 9α-fluorohydrocortisone only slightly suppressed the excretion of all the individual corticosteroids.

Received August 21, 1957.

* Supported by grants from the National Research Council of Canada, the Banting Research Foundation and the Lilly Research Laboratories, Indianapolis, Ind.

INVESTIGATIONS regarding the excretion and identification of adrenocortical metabolites in the urine of patients with adrenal dysfunction have contributed much to our understanding of the etiology of the various forms of adrenal disease. In the following study the excretion patterns of α,β-unsaturated and α-ketolic corticoids have been determined in patients with Cushing's syndrome, Addison's disease and the adrenogenital syndrome. Patterns were determined in the urine of patients in the untreated state, and during treatment with ACTH, cortisone or 9α-fluorohydrocortisone, both before and after adrenalectomy. Similar studies were carried out in patients with panhypopituitarism and congenital adrenal hyperplasia.

METHODS

Urinary corticosteroid patterns

(a) *Hydrolysis and extraction*

A 12-hour aliquot of a 24-hour collection of urine was used. The urine was first extracted with chloroform to remove the free corticosteroids. Following this the urine was incubated with β-glucuronidase[1] (300 units per ml. of urine) at pH5 and 37° C. for forty-eight hours. The corticosteroids liberated by this procedure were extracted with chloroform. The urine was then acidified to pH1 and re-extracted with chloroform. The 3 chloroform extracts were combined and were washed with 0.1 *N* NaOH and water. After evaporation of the solvent, the residue was dissolved in small amounts of alcohol and was applied on the paper strip.

(b) *Chromatography*

Chromatography of the corticosteroids was carried out according to the method of Burton, Zaffaroni and Keutmann (1). An attempt was made to obtain a pattern of the α-ketolic and the α,β-unsaturated corticosteroids ranging in polarity from 11-dehydrocorticosterone to tetrahydrocortisol. In order to obtain good resolution of the corticosteroids present in the urinary extract, the procedure outlined in the flow sheet in Figure 1 was used. The steroids were finally separated according to their polarity on three paper strips, No. 1 containing the most polar compounds, and No. 2 and No. 3 containing corticosteroids of decreasing polarity. The extract was initially purified by a 48-hour development in the benzene-formamide system (B-F). The effluent which ran off the paper during the first five hours contained most of the urinary pigments. As no corticosteroids were present in this fraction, it was discarded. The effluent from five to forty-eight hours still contained urinary pigments. Purification was accomplished by either chromatography on silica-gel columns (2), six-hour development in the undiluted toluene-propyleneglycol system (TP), or development in the water-heptanol system of Staudinger (3) for four hours. The latter method was preferred. In this system the pigments were retained at the starting line and could be separated from the corticosteroids which moved down the paper. The corticosteroids were eluted from this paper and were later applied to paper No. 3. To obtain the final papers, No. 1 and No. 2, equal aliquots from the eluate of the benzene-formamide paper chromatogram were applied to papers impregnated

[1] Ketodase (Warner-Chilcott).

with a mixture of methanol-propylene-glycol (1:1) and run for seven and three days respectively in the toluene-propylene-glycol system. The overflow from paper No. 2 was combined with a corresponding aliquot of the eluate from the heptanol chromatogram and was then applied for the final chromatogram No. 3 to the starting line of a paper impregnated with undiluted propylene-glycol. This paper strip was developed for twenty-four hours in the toluene-propylene-glycol system. In the 3 final paper chromatograms, a 0.8-hour aliquot of urinary extract per centimeter width of paper was applied, with the exception of the extracts from the 2 cases of adrenal hypofunction. Extracts of urine obtained from the same patient during various periods of treatment were run on separate strips of the same paper chromatogram. This method allowed a good comparison of the patterns obtained under different conditions, since it eliminated most of the variations which are frequently observed in chromatographic mobility. A satisfactory separation of tetrahydrocortisol, tetrahydrocortisone, and cortisol was generally obtained on paper No. 1, whereas on paper No. 2 these three compounds were not properly resolved; but cortisone and tetrahydrocorticosterone were retained on the paper and well separated. Only this latter part of paper No. 2 is shown in the various patterns. The compounds allo-pregnane-3α,11β,21-triol-20-one (allo-THB), pregnane-3α,21-diol-11,20-dione (THA), 11-desoxycortisol, corticosterone and 11-dehydrocorticosterone were found on paper No. 3.

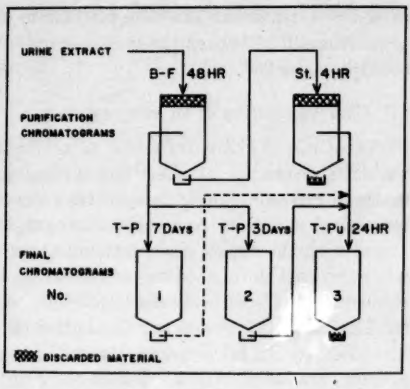

FIG. 1. Flow sheet for the chromatography of urinary corticosteroid extracts.

The foregoing procedure was used for chromatography of extracts in which larger than normal amounts of corticosteroids were expected. For urinary extracts which contained smaller amounts, such as those obtained from the urine of normal subjects and from patients with Addison's disease and hypopituitarism, paper No. 2 was not required. In these instances the material was run for five to seven days in the toluene-propylene-glycol system, and the effluent from this paper was combined with the eluate of the heptanol chromatogram and directly applied to paper No. 3.

(c) *Scanning of paper chromatograms Nos. 1, 2 and 3*

For the detection of corticosteroids containing a Δ^4,3-keto grouping in ring A, strips of the paper chromatograms 1.0 cm. wide were scanned every 0.5 cm. in a photometer at a wave length of 240 mμ. The optical densities were plotted against the distance from the starting line. The curves thus obtained are shown by the solid line in the figures.

For the detection of the α-ketolic steroids the formazans of these compounds were developed on the same strips by dipping them in a freshly prepared mixture of 2 per cent blue tetrazolium (BT), 10 per cent aqueous NaOH and 60 per cent aqueous methanol (2:3:3) and washing them with 2 *N* sulfuric acid and water. After drying at 80° C. the strips were scanned in a similar fashion at 540 mμ. These materials are represented by the grey areas in the figures.

Though the patterns provide an approximate estimation of the amount of a com-

pound present, it has not been possible to develop the method in such a way that strict proportionality between the area covered by the curve and the amount of steroid present could be achieved.

(d) *Characterization of the compounds*

As an initial characterization of a compound, the mobility was considered together with the properties of absorbing ultraviolet light and reducing blue tetrazolium. The material remaining on the paper after the scanning strip was cut off was eluted for each individual spot and was rechromatographed (sometimes repeatedly), either alone or mixed with the appropriate authentic compound, to establish its true rate of flow. This was expressed in relation to cortisone ($R_E = 1.00$). Acetate formation and the spectra in concentrated H_2SO_4 were also used for the characterization of compounds as suggested by Burton and coworkers (1). Tetrahydro-11-desoxycortisol from Patient F.T. was identified by its infra-red spectrum. The steroids which were observed in the urinary extracts are listed in Table 1 with some of their chemical characteristics, in the order of

TABLE 1. PROPERTIES OF STEROIDS ISOLATED FROM URINE

Abbreviation	Steroid	R_f in T-P*	UV absorpt. max. (mμ.)	BT reduction	PS reaction	λ Max. of H_2SO_4 spectra
X_1	Unidentified	0.15–0.18	240	–	–	
THF	Pregnane-3α,11β,17α,21-tetrol-20-one	0.16–0.18	none	+	+	330.415.510
THE	Pregnane-3α,17α,21-triol-11,20-dione	0.26–0.28	none	+	+	330–335.405–410
F	Cortisol	0.45–0.49	242	+	+	285.380–395 460–475.
X_2	Unidentified	0.80	none	+	?	
X_3	Unidentified	0.78–0.81	240	–	–	
X_4	Unidentified	0.81	238	–	–	
THS	Pregnane-3α,17α,21-triol-20-one	0.98–1.01†	none	+	+	315.410
E	Cortisone	1.00	238	+	+	285.343.415
THB	Pregnane-3α,11β,21-triol-20-one	1.74–1.79	none	+	–	310.415
DHE	Pregnane-17α,21-diol-3,11,20-trione	1.79	none	+	+	
Allo THB	Allopregnane-3α,11β,21-triol-20-one	2.74–2.96	none	+	–	320.410
THA	Pregnane-3α,21-diol-11,20-dione	3.36–3.58	none	+	–	280.350.415
X_5	Unidentified	2.94–3.56		–	–	
S	Δ^4-Pregnene-17α,21-diol-3,20-dione	3.67–3.81	240	+	+	290.340.535
B	Corticosterone	5.12–5.36	242	+	–	285.330.375.455
X_{10}	Unidentified	4.27–5.72		–	–	
X_{11}	Unidentified	8.80–9.57		–	–	
A	11-Dehydrocorticosterone (not found)	13.50	239	+	–	280.355.415

* Toluene-propyl-glycol system. These values were obtained after re-chromatography of the individual spots either alone or as mixed chromatograms, and are expressed in relation to cortisone mobility = 1.00.

† R_f in BF system for THS = 3.9 when E = 1.0.

decreasing polarity. The identity of a number of compounds observed repeatedly in the patterns could not be established. These are listed as Compounds X_1 to X_{11}.

Endocrine assays

The total urinary 17,21-dihydroxy-20-ketosteroids were measured by a modification of the Silber-Porter reaction (4) after incubation of the urine with β-glucuronidase (normal range 2.0–8.6 mg. per twenty-four hours). In Patient G.A. the corticosteroids were determined as formaldehydogenic steroids by the method of Daughaday *et al.* (5), (nor-

mal range 0.4–0.8 mg. per twenty-four hours). Urinary 17-ketosteroids were determined by the procedure of Callow *et al.* (6), and urinary aldosterone by bioassay (7). Urinary pregnanediol and pregnanetriol were measured in Patient F.T. by the method of Bongiovanni and Clayton (8).

ACTH test

To investigate the responsiveness of adrenal function to corticotropin, a standard ACTH test was performed on all patients. A dose of 20 units of ACTH[2] was administered intramuscularly every six hours, starting at 8:00 A.M., over a period of forty-eight hours. Immediately before the first injection, and four hours and forty-eight hours afterward, blood samples were taken and the plasma was assayed for corticosteroids by means of a modification of the Silber-Porter method (4). Urinary corticoids were also determined and an aliquot of the urine collected during the administration of ACTH was used for the corticosteroid patterns. In 2 of the earlier patients (G.A. and D.M.) the ACTH test was performed using a dose of 25 units of ACTH (Armour) every six hours.

CLINICAL MATERIAL

Normal subjects

Urine collections from 3 healthy persons, 34–38 years of age (1 male and 2 females), covered periods when they were carrying out their usual work.

Cushing's syndrome

S.K., a 60-year-old woman with Cushing's syndrome of eight or nine years' duration, exhibited the classic features of this disease. The clinical findings included minimal virilization, normal blood pressure, a diabetic glucose tolerance curve, normal serum electrolyte levels and marked generalized osteoporosis. Urinary 17-ketosteroids were 25.5 mg., and corticosteroids 20.4 mg. per twenty-four hours. During administration of ACTH, the 17-ketosteroids rose to 43.5 mg., and the corticosteroids to 38.3 mg. per twenty-four hours. The level of plasma corticoids was 22 μg. per 100 ml., and increased to 69 μg. at four hours and to 183 μg. at the end of forty-eight hours of ACTH administration. Urinary aldosterone on two occasions was within the normal range (1.6 and 2.3 μg. per twenty-four hours), and on another day was slightly elevated at 8.7 μg. per twenty-four hours. A one-stage total adrenalectomy was performed; at operation bilateral adrenocortical hyperplasia was evident. Preoperatively a total dosage of 700 mg. of cortisone acetate was given over a three-day period. Following operation the cortisone dosage was gradually reduced to 50 mg. per day. At this stage the 24-hour excretion of 17-ketosteroids was 4.3 mg., of corticoids 9.2 mg., and of aldosterone <1.0 μg.

G.A., a 27-year-old woman with Cushing's syndrome of two to three years' duration, had moderate virilization, hypertension (190/140 mm. Hg), diabetes, normal levels of serum sodium and chloride, a low level of serum potassium (3.8 mEq./L.), and moderate osteoporosis. The 24-hour excretion of 17-ketosteroids was 35 mg., and of formaldehydogenic steroids (FS) 2.64 mg. Following administration of 100 units of ACTH (Armour) daily for two days, the urinary 17-ketosteroids increased to 101.0 mg. and the FS to 16.5 mg. per twenty-four hours. Aldosterone output was slightly above the normal range (7.9 μg. per twenty-four hours). Total adrenalectomy was performed in two stages with an interval of ten days between operations. Examination of the adrenals revealed bilateral cortical hyperplasia. Prior to the removal of the first adrenal, cortisone (800 mg.)

[2] Acton-X (Nordic Biochemicals).

was administered over a three-day period. In the interval between the first and second adrenalectomy the cortisone dosage was gradually reduced. During this period urine was collected for pattern C. Eighteen days following the second adrenalectomy the symptoms of diabetes and hypertension had disappeared (blood pressure 120/80). The patient was maintained on 37.5 mg. of cortisone daily, and excreted normal amounts of FS corticoids and 17-ketosteroids (0.81 and 1.0 mg. per twenty-four hours, respectively). No aldosterone could be detected in the urine at this time.

Adrenocortical hypofunction

K.W., a 53-year-old man, had Addison's disease secondary to tuberculosis of the adrenal glands. Outstanding manifestations were muscular weakness, and pigmentation of the mucous membranes and cutaneous pressure areas of the body. His blood pressure was 95/65. The level of serum sodium was 133 mEq., chloride 95.4 mEq. and potassium 4.4 mEq. per liter. He had suffered occasional attacks of high fever with peripheral vascular collapse and low blood pressure readings; these attacks could be relieved by the administration of intravenous glucose-saline and hydrocortisone. On admission it was not possible to detect any urinary corticoids (Silber-Porter method) and 17-ketosteroids were 0.2 mg. per twenty-four hours. The response to ACTH was minimal, the corticoids being 0.06 mg., and 17-ketosteroids 1.4 mg. per twenty-four hours. The plasma corticoid level rose from 0 to 0.8 μg. per 100 ml. after four hours, and to 4.5 μg. per 100 ml. after forty-eight hours of ACTH administration. The patient improved during treatment with 2 mg. of 9α-fluorohydrocortisone per day. At the time of discharge he was receiving 25 mg. of cortisone and 3–4 Gm. of sodium chloride daily.

D.M., a 52-year-old woman, had moderate hypopituitarism secondary to a suspected pituitary tumor which had been treated with deep x-ray therapy eight years previously. Rheumatoid arthritis had developed a year before the present study. The clinical findings included obesity, reduced body hair, sparse axillary and pubic hair, a flat glucose tolerance curve, a basal metabolic rate within normal limits and normal levels of serum electrolytes. Her blood pressure fluctuated between 160/100 and 230/120. She received cortisone, thyroid and estrogen as replacement therapy. Cortisone was withdrawn four days prior to the collection of urine for the study of the steroid excretion pattern. At this time 17-ketosteroids were 0.1 mg., and corticosteroids 1.4 mg. per twenty-four hours. A dosage of 100 mg. of ACTH (Armour) administered daily for four days did not stimulate the adrenals. Urinary 17-ketosteroids were 1.4 mg., and corticosteroids 2.0 mg. per twenty-four hours. Aldosterone excretion was 9.1 and 5.8 μg. per twenty-four hours.

Virilizing adrenal hyperplasia

F.T.,[3] a 14-year-old boy, was suffering from congenital adrenal hyperplasia associated with episodes of hypertension and severe headaches. Between the hypertensive attacks the blood pressure was 130/90. At 9 years of age the child showed signs of premature masculinization with hirsutism and advanced development of muscles and bones. On admission the urinary 17-ketosteroids ranged from 21.9 to 48.1 mg., and the 17-ketogenic steroids from 55 to 93 mg. per twenty-four hours. The 24-hour excretion of aldosterone was 3.1 μg.; of pregnanetriol, 1.70 mg.; and of pregnanediol, 3.95 mg. Cortisone was administered in a dosage of 50 mg. daily, and the 17-ketosteroids decreased to 4.1 mg., and the 17-ketogenic steroids to 11.2 mg. per twenty-four hours.

F.L., a 21-year-old girl with the adrenogenital syndrome, showed signs of adreno-

[3] A patient of Dr. Frances McCall (Montreal Children's Hospital). The clinical history and findings will be published elsewhere by Dr. McCall.

cortical hyperfunction with virilization—hirsutism, obesity, moderate facial acne and irregular menstrual periods. The blood pressure was 140/80, and the glucose tolerance curve and the serum electrolyte levels were normal. The 24-hour urinary 17-ketosteroids were 46.5 mg., and the corticosteroids (8.9 mg.) slightly above the normal range. During administration of ACTH both types of urinary steroids increased greatly to 96.0 and 30.1 mg. per twenty-four hours, respectively. The plasma corticoid level rose from 10 to 35 and 55 μg. per 100 ml. With the administration of 2 mg. of 9α-fluorohydrocortisone daily for five days, a transient suppression of urinary 17-ketosteroids to 8.1 mg. per twenty-four hours was achieved.

RESULTS

Normal subjects

Fractionation of the urinary corticosteroids in the 3 normal persons (1 man and 2 women) showed similar steroidal patterns (Fig. 2). Small amounts of the biologically active hormones, cortisol and cortisone, were

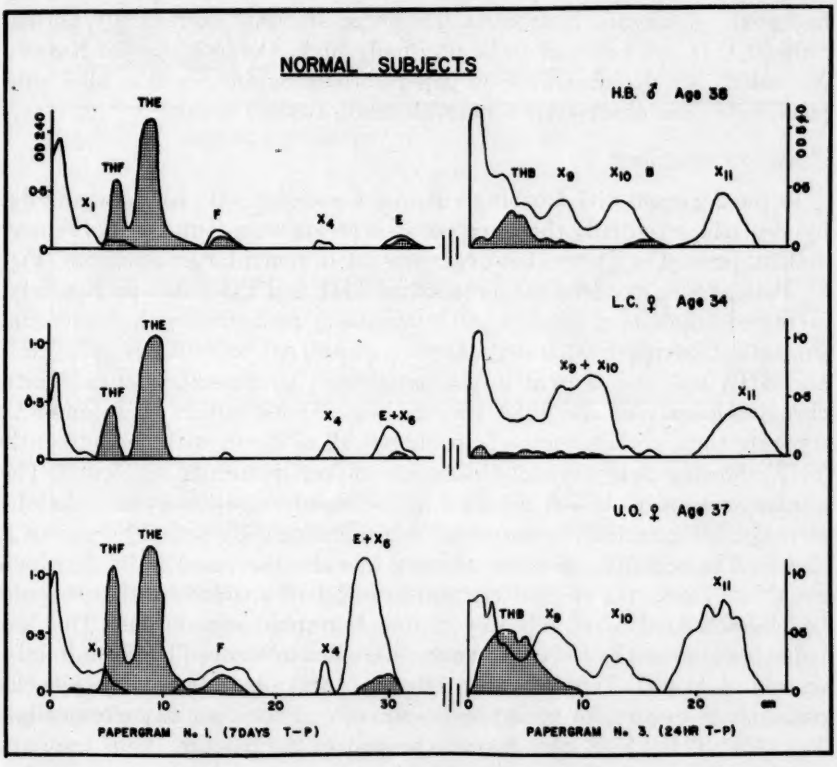

FIG. 2. *Corticosteroid excretion patterns of normal subjects.* The shaded areas represent the α-ketolic, and the unshaded areas under the solid line, the α,β-unsaturated steroids. Abbreviations are as described in Table 1. Aliquot of urinary extract applied = 0.8 hr./cm.

found in the urine of 2 of these subjects, whereas in the third only cortisone was demonstrable. Large amounts of tetrahydrocortisol (THF) and tetrahydrocortisone (THE), metabolites of compounds F and E, were present in all 3 patterns. Small amounts of corticosterone were found only in the extracts of urine from the male subject and 11-dehydrocorticosterone was absent from all 3 extracts. Compound THB, a metabolite of corticosterone, was excreted by one of the female subjects and by the male subject, in amounts of the same order as those of THF and THE, but the other female subject excreted only traces of this steroid. A series of unidentified compounds, X_1, X_4, X_6, X_9, X_{10} and X_{11}, absorbing light in the ultraviolet region, were also excreted. These substances did not reduce blue tetrazolium. Compounds X_1 and X_4 appeared to be of minor importance. Compound X_6, with a running rate in toluene-propylene glycol similar to that of cortisone, has been frequently observed in various urines. It is usually liberated by enzymic hydrolysis. The large amounts excreted by normal Subject U.O. are believed to be unusually high. Compounds X_9, X_{10} and X_{11}, which are demonstrated on paper chromatogram No. 3 in all 3 subjects, have been observed in almost all urines studied so far.

Cushing's syndrome

In the 2 women with Cushing's disease associated with bilateral adrenal hyperplasia, essentially the same corticosteroids were found as in the normal subjects. They were, however, present in much larger amounts (Fig. 3). Patient S.K. excreted the metabolites THF and THE and, particularly, increased amounts of cortisol and cortisone as compared with the normal subjects. Less polar α-ketolic material, considered to be THB, allo-THB and THA was also present in the patterns in increased amounts. Mixed chromatography of the THA fraction was carried out in an attempt to separate some corticosterone from it, but all of it ran with the authentic THA, showing no ultraviolet absorption; no corticosterone was found. The administration of ACTH resulted in increased excretion of all α-ketolic steroids, the greatest rise occurring in the biologically active hormones F and E. The excretion of corticosterone was also increased to levels which could be observed in the pattern and in sufficient amounts so that it could be characterized after repeated chromatographic separations. The less polar compounds X_9 to X_{11}, however, were not influenced by the administration of ACTH. Ten days after bilateral total adrenalectomy, while the patient was being maintained on 50 mg. of cortisone per day, compounds X_1, THF, THE, F, E and X_6 were present in the pattern. Thus they appeared to be metabolites of the administered cortisone. As compounds X_9, X_{10} and X_{11} were not excreted after adrenalectomy, it is suggested that

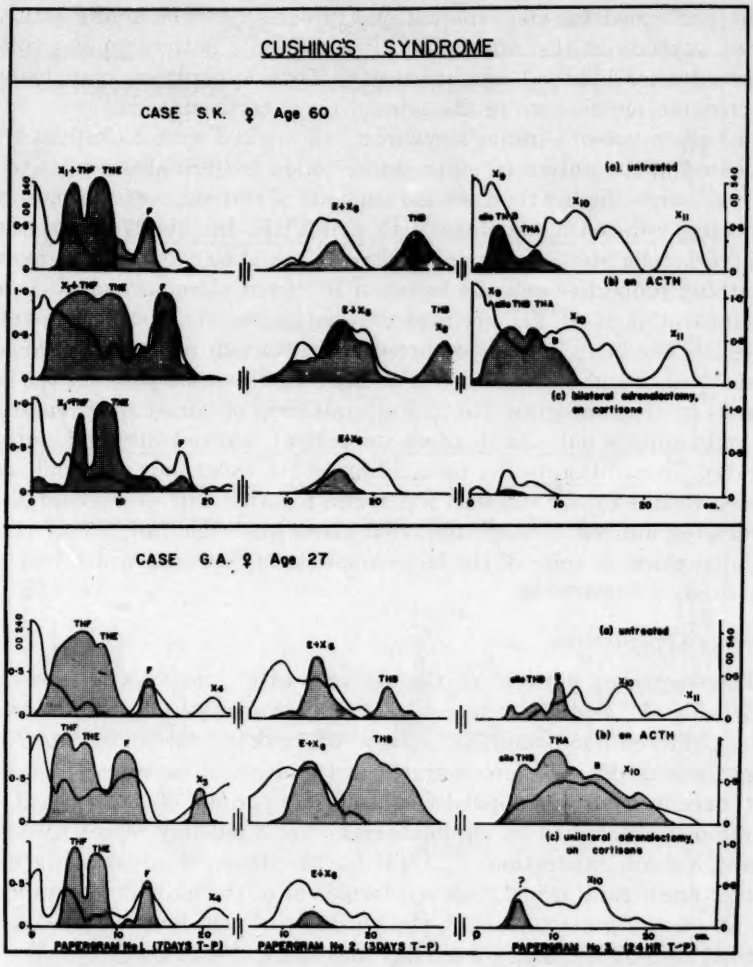
CUSHING'S SYNDROME
CASE S.K. ♀ Age 60
(a). untreated
(b) on ACTH
(c) bilateral adrenalectomy, on cortisone
CASE G.A. ♀ Age 27
(a) untreated
(b) on ACTH
(c) unilateral adrenalectomy, on cortisone
PAPERGRAM No 1. (7DAYS T-P)
PAPERGRAM No 2. (3DAYS T-P)
PAPERGRAM No 3. (24 HR T-P)

FIG. 3. *Corticosteroid excretion patterns of patients with Cushing's syndrome.* Abbreviations as described in Table 1. Aliquot of urinary extract applied = 0.8 hr./cm.

they must be of adrenal origin. The trace of reducing material on paper No. 3 might possibly represent some dihydrocortisone.

In another patient with a similar clinical picture (not included in this study) a very similar set of corticosteroid excretion patterns was obtained during the period of no treatment, during ACTH administration, and after bilateral adrenalectomy. Shortly afterwards hypophysectomy had

to be performed for the removal of a progressively enlarging pituitary tumor. A study carried out at this time showed a pattern similar to that observed after bilateral adrenalectomy. Thus hypophysectomy had not affected the metabolism of the administered cortisone.

The other case of Cushing's syndrome shown in Figure 3 (Patient G.A.) presented in the untreated state, an excretion pattern also similar to the previous ones—higher than normal amounts of cortisol, cortisone and their respective reduced metabolites THF and THE. In this patient the least polar α-ketolic steroid was corticosterone, as shown by the ultraviolet-absorbing properties and the behavior in mixed chromatograms. During administration of ACTH, she also excreted increased amounts of α-ketolic material. The main increase occurred in the steroids present on chromatograms Nos. 2 and 3, whereas in Patient S.K. this took place in the compounds on chromatogram No. 1. Pattern C was obtained after removal of the right adrenal only. At this time the patient received 50 mg. of cortisone per day. In addition to the metabolites of the exogenous cortisone, some unidentified α-ketolic material was found together with compound X_{10} on chromatogram No. 3, indicating that there was some function of the remaining gland in spite of the large amounts of cortisone which had been previously administered.

Adrenal hypofunction

The excretion pattern of the patient with Addison's disease (Mr. K.W., Fig. 4), aside from traces of tetrahydrocortisol and tetrahydrocortisone, showed compound X_6 as the only peak on chromatogram No. 1. There was no F or E demonstrable in the urine, even when three times the concentration was applied (*i.e.* 2.4 hours per cm. of paper strip). The ultraviolet absorption on chromatogram No. 3 possibly represented compound X_9. Administration of ACTH for two days did not raise the excretion of crude total urinary corticosteroids or of the individual compounds shown on the pattern, though the plasma corticoid level responded very slightly to ACTH, rising from 0 to a maximum of 4.5 μg. per 100 ml.—still within the range of low-normal values.

While untreated, the patient with panhypopituitarism (Mrs. D.M., Fig. 4) exhibited a urinary steroid pattern qualitatively similar to those obtained in the normal subjects, although the amounts excreted were considerably less. The concentration of application was more than twice (1.9 hours/cm.) the usual one. Compounds THF, THE, F, E and some less polar α-ketolic material (the identity of which is not established), as well as compounds X_9 to X_{11} were present. The administration of ACTH left the excretion pattern virtually unchanged. Only cortisone showed a slight rise.

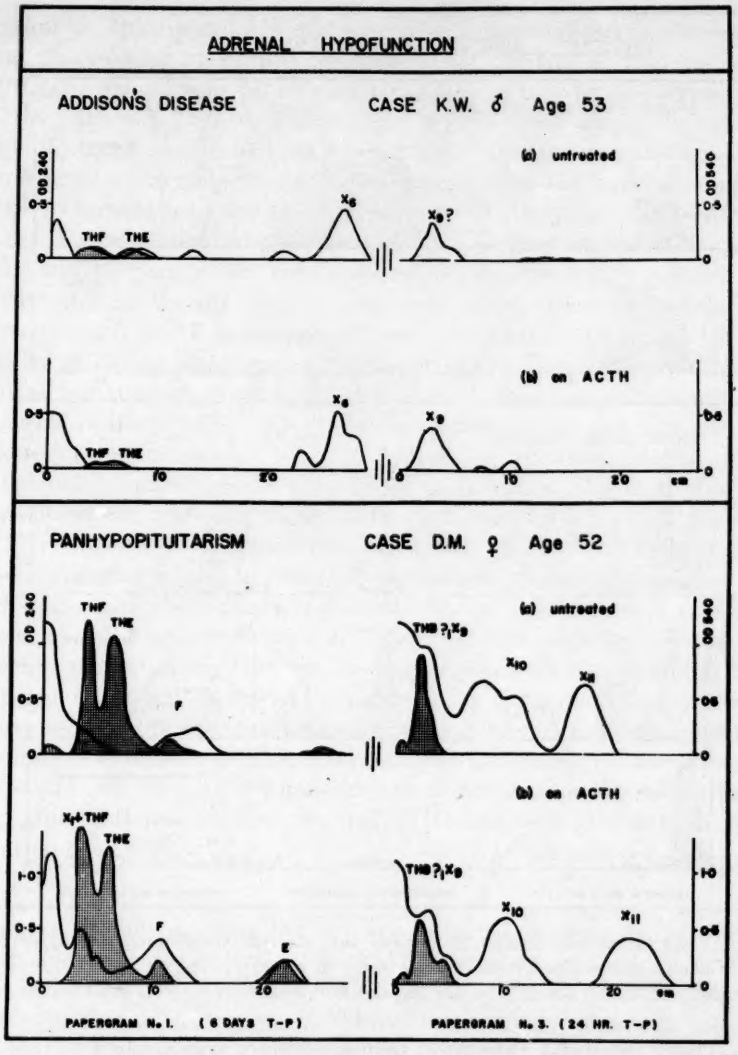

FIG. 4. *Corticosteroid excretion patterns of patients with adrenal hypofunction.* Abbreviations as described in Table 1. Aliquot of urinary extract applied = 0.8 hr./cm. for Patient K.W. and 1.9 hr./cm. for Patient D.M.

Virilizing adrenal hyperplasia

The boy with congenital adrenal hyperplasia (F.T., Fig. 5) was the only patient in whom a qualitatively different corticoid excretion pattern was

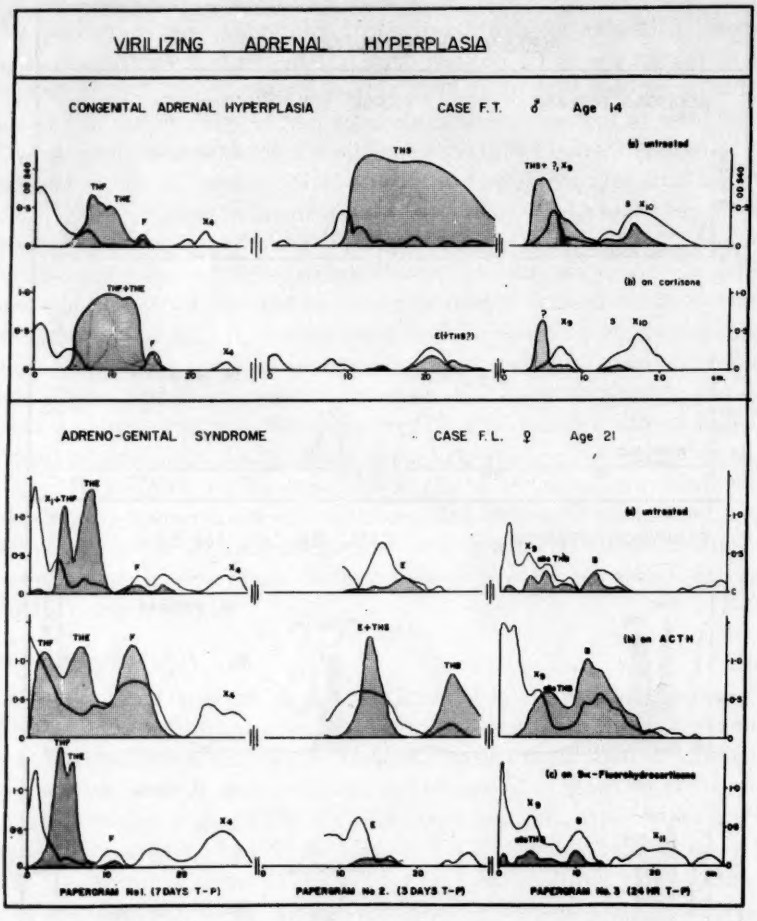

FIG. 5. *Corticosteroid excretion patterns of patients with virilizing adrenal hyperplasia.* Abbreviations as described in Table 1. Aliquot of urinary extract applied = 1.6 hr./cm, for Patient F.T., and 0.8 hr./cm. for Patient F.L.

seen. Twice the usual amount of urinary extract was applied in this case but the pattern on chromatogram No. 1 revealed only small amounts of compounds THF, THE and cortisol. Large amounts of an α-ketolic material were, however, found on chromatogram No. 2 in the region of cortisone. Some of it ran off into the overflow and contributed to the first peak on chromatogram No. 3. This material did not absorb ultraviolet light. The substance ran with THS in a mixed chromatogram, formed an acetate with a mobility equal to that of THS-acetate, and showed in sulfuric-acid

maxima at 315 mμ and 510 mμ. After re-chromatography in the B-F system, the infrared spectrum[4] established the identity of this material as THS. The second peak on chromatogram No. 3 represented compound S, on the basis of a positive Porter-Silber reaction and mixed chromatography. Whether small amounts of cortisone were present in this extract could not be ascertained, since they were lost in the bulk of compound THS. When cortisone was administered to the patient, the excretion of THS was almost completely suppressed and larger amounts of compounds THF and THE appeared, thus showing a normal excretion pattern.

Patient F.L., the girl with the adrenogenital syndrome did not show any obvious abnormality in urinary corticosteroid excretion (Fig. 5). On chromatogram No. 3 an appreciable amount of corticosterone was present. During administration of ACTH a pattern was obtained similar to that observed in Patient G.A. with Cushing's syndrome, in so far as there was a considerable increase not only in cortisol and cortisone output but also in the output of corticosterone and its metabolites THB and allo-THB. The spot in the cortisone region was investigated for a possible admixture of THS. After re-chromatography in the B-F system, four-fifths of the α-ketolic material proved to be THS, whereas only one-fifth was found to be cortisone. Since the region of cortisol in the pattern showed a similar appearance of a higher peak with BT-scanning than with the UV-light, the possibility is considered that in this region also a second substance might be present. After three days of administration of 9α-fluorohydrocortisone, suppression of the adrenals became apparent by the obviously decreased amounts of compounds THF, THE and the biologically active hormones, E, F and B. In the patient's untreated state and after suppression therapy, only allo-THB was noticed. Normal THB was seen only during administration of ACTH.

DISCUSSION

In the normal subjects the urinary patterns showed the presence of compounds tetrahydrocortisol, tetrahydrocortisone, cortisol, X_4 and cortisone. Corticosterone was detected in only 1 of the subjects and tetrahydrocorticosterone in 2 subjects. Three unidentified compounds, X_9, X_{10} and X_{11}, which absorbed ultraviolet light, were also observed. The presence of cortisone and cortisol in normal urines has been well established (9–11). Schneider (9) found a level of 66 μg. of cortisone per liter and Burton *et al.* (1) estimated by means of chromatographic methods the amounts of cortisone and cortisol to be in the order of 15–40 μg. per day. Tetrahydrocortisone was isolated from the β-glucuronidase hydrolyzed fraction of nor-

[4] Identification was carried out by Dr. Heard and Mr. Zaharia in the Biochemical Department of McGill University.

mal urine by Schneider (9). Gray (12) reported amounts from 200–4000 μg. per twenty-four hours for the sum of THE plus THF. The predominance of THE over THF which is demonstrated in our figures is in agreement with other findings of Gray, de Courcy and coworkers (13, 14). These authors observed also some faster moving α-ketolic material in the urine of normal and pregnant women, which they termed R_5, R_6 and R_7. In more recent investigations, Touchstone *et al.* (15) identified α-ketolic material in this region of the chromatograms as THB, allo-THB and THA. Dihydrocortisone (pregnane-17α,21-diol-3,11,20-trione), which has been detected in urine by Schneider (16), may also contribute to the material found in this region. However, since the metabolic reduction of the dihydro compounds to the tetrahydro derivatives normally occurs very rapidly (17), it can account for only a small amount. The adrenal origin of the 3 compounds, X_9, X_{10} and X_{11}, is suggested by the fact that they disappeared from the urinary patterns after bilateral adrenalectomy. Compound X_9 showed an ultraviolet absorption maximum at 240 mμ, characteristic of an α,β-unsaturated steroid. Compounds X_{10} and X_{11} were also absent from the urines of the patient with Addison's disease and diminished in the urine of the patient with the adrenogenital syndrome.

The corticosteroids excreted by the 3 untreated patients with Cushing's disease due to bilateral adrenal hyperplasia were similar to those observed in the normal subjects. However, the amounts of all α-ketols were strikingly increased, the greatest elevation involving the active hormones cortisol and cortisone. In Patient G.A., corticosterone also was greatly increased. The adrenal gland of this patient (first operation) secreted compounds F, E and B in incubation experiments carried out by Heard and associates (18). These 3 hormones, together with their corresponding metabolites, THF, THE, THB and allo-THB (which are reduced in ring A), were also excreted in the urine (Fig. 3, G.A.a). The second adrenal, which was removed ten days after the first operation, secreted only small amounts of compound F during the incubation, so that it may be considered that the urinary corticosteroids excreted at this time were derived mainly from the metabolism of the administered cortisone (Fig. 3, G.A.c). Whether these findings indicate that both glands were functionally different initially or whether the second one behaved differently because of cortisone suppression, cannot be decided. The presence of UV-absorbing and α-ketolic material in the urinary pattern indicated some secretory activity of the second adrenal at the time of operation, in spite of the administered cortisone. Although no corticosterone could be demonstrated in the urinary pattern of the other patient with Cushing's disease, relatively large amounts of compounds THB, allo-THB and THA were present (Fig. 3, S.K.a). The presence of the latter substances could be explained by the findings of Heard (19) who observed compounds F and B in approximately

equal amounts in the extract from the adrenal glands of this patient. A general elevation of all BT-reducing corticosteroids in patients with Cushing's disease has also been shown by Gray (12) and by Dohan *et al.* (20), whereas Wilson *et al.* (21), by means of evaluating the paper eluates with the formaldehydogenic and the Porter-Silber method, found five times the normal value for the $C_{21}O_5$-steroids and only about twice the normal value for the $C_{21}O_4$-steroids.

The patient with moderate panhypopituitarism excreted the normally occurring corticosteroids, although at a low level, and so did a patient with severe panhypopituitarism. Richardson and coworkers (22), however, could demonstrate the absence of corticosteroids in such a case when treatment was withdrawn.

None of the active hormones was observed in the urine of the Addisonian patient, which is in agreement with the findings of Gray (12), who also found occasional traces of THF or THE, or both. Burton *et al.* (23) were able to detect some cortisol and cortisone in 2 patients with long-standing Addison's disease. The presence of compound X_6, a metabolite of cortisone, and of compound X_9 in these urines was puzzling. Possibly these compounds have a very slow renal clearance, so that they are demonstrable for several days after withdrawal of cortisone therapy.

The only patient with a qualitatively different urinary pattern was the boy with congenital adrenal hyperplasia. In this case, compound THS was the corticosteroid excreted in the largest amount. The predominance of this compound in the urine of such a patient was shown earlier by Eberlein and Bongiovanni (24), together with the presence of 3α,21-dihydroxy-pregnane-20-one (TH-DOC) and 3α,17α,20,21-tetrahydroxy-pregnane. With the report of the absence of cortisol and cortisone and the presence of compound S in the blood of their patient, the observations of these authors lend further support to the hypothesis of a disturbed synthesis of cortisol by the adrenals of patients with congenital adrenal hyperplasia (25–28). It was shown earlier (29–31) that in these patients an increase occurs in urinary pregnane-3α,20α-diol and pregnane-3α,17α,20α-triol—metabolites considered to be the excretory products of precursors, such as progesterone and/or 17α-hydroxyprogesterone, which accumulate due to the failure of certain hydroxylating enzyme systems in these cases. In Patient F.T. considerable amounts of pregnanediol (3.95 mg. per twenty-four hours) and lesser amounts of pregnanetriol (1.69 mg. per twenty-four hours) were found. There was no indication of the presence of TH-DOC in the corticosteroid pattern of this patient and 3α,17α,20α,21-tetrahydroxy pregnane, if present, would not have been observed by the technique used in this study.

Touchstone *et al.* (32) reported in a case with virilizing adrenal tumor, a relatively large output of compound THS in the urine. Our Patient F.L.,

in whom the diagnosis of virilizing adrenal hyperplasia was confirmed at operation, excreted this compound only during the administration of ACTH.

The administration of ACTH to the patients with Cushing's disease resulted in a further increase of the already elevated α-ketolic steroids. It was interesting to note that in 2 patients the main increase occurred in the $C_{21}O_5$-steroids, and in the third patient in the $C_{21}O_4$-steroids. Unfortunately we did not have similar studies with ACTH on normal subjects for comparison. However, the investigations of Dohan *et al.* (20) indicated general increases in all α-ketolic compounds of the $C_{21}O_5$ and the $C_{21}O_4$ series in normal subjects following administration of ACTH.

It was not possible to withdraw therapy from the totally adrenalectomized patients during our studies, so the urinary patterns in these subjects were obtained while they received small amounts of cortisone. Thus the corticosteroids excreted at this stage are considered to be the metabolic products of the administered cortisone; they included compounds X_1, THF, THE, F, E, X_6 and possibly DHE. These findings are in agreement with the results of Burton *et al.* (23), Burstein *et al.* (33) and Gray and Lunnon (34), who showed the same α-ketolic corticosteroids as being urinary metabolites of administered cortisone. This fact implies the metabolic interconvertibility of cortisone and cortisol, a process for which there is much evidence at the present time.

Another metabolite of cortisone, 3α,17α-dihydroxy-pregnane-11,20-dione, was isolated by Lieberman *et al.* (35) from urines after the administration of cortisone. This substance belongs to the group of C_{21}-desoxy-corticosteroids which would not be demonstrated by the procedure used in the development of the patterns in this study, just as substances belonging to the group of C_{20}-hydroxy metabolites were not shown by this technique. That such compounds constitute a large proportion of the metabolites of adrenocortical hormones was shown by Fukushima *et al.* (36), who isolated the 20α and 20β forms of THF and THE from the urine of a patient with Hodgkin's disease after administration of ACTH.

Acknowledgments

We wish to thank Dr. Frances McCall of the Montreal Children's Hospital for permission to use the studies on Patient F.T., and Dr. C. J. Pattee for the material and information on Patient D.M. We are indebted to Dr. Heard and Mr. Zaharia for the infrared spectrum data and the identification of pregnane-3α,17α,21-triol-20-one. The technical assistance of Mrs. Eleanor Wallace is also gratefully acknowledged.

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EFFECT OF PREDNISONE ON ADRENAL RESPONSIVENESS TO CORTICOTROPIN IN NORMAL SUBJECTS AND IN PATIENTS WITH TREATED AND UNTREATED CUSHING'S SYNDROME

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ABSTRACT

A study of the adrenocortical response to the injection of corticotropin before and following the daily oral administration of 40 mg. of prednisone for seven or more days has been made in 13 normal subjects, in 2 patients with active untreated non-tumorous Cushing's syndrome, and in 3 patients with partial remission and in 4 patients with complete remission of the disease following therapy. The level of plasma 17-hydroxycorticoids was used as the index of adrenocortical response. In the subjects without evidence of adrenocortical hyperfunction, a significant suppression of the adrenocortical response to corticotropin was uniformly noted following the administration of prednisone. In the 2 patients with active, untreated Cushing's syndrome due to bilateral adrenocortical hyperfunction there was no suppression of the adrenocortical response to corticotropin under the conditions of the prednisone suppression test. Studies conducted in the 7 cases of non-tumorous Cushing's syndrome, in partial or in complete clinical and laboratory remission following irradiation of the pituitary, alone or combined with unilateral adrenalectomy, revealed the following: In 1 case of partial remission and 2 cases of complete remission, normal suppression of the adrenocortical response to corticotropin followed the administration of prednisone. In 2 cases of partial remission and in 2 cases of complete remission this suppression did not occur.

INTRODUCTION

RECENT studies have demonstrated that the daily administration of 30 to 50 mg. of prednisone to normal subjects for a seven-day period results in suppression of the adrenocortical response to corticotropin (ACTH) as measured by changes in the level of the plasma 17-hydroxycorticoids (1). This is the basis of the prednisone suppression test, and is

Received July 19, 1957.

* Moritz Rosenthal Fellow in Endocrinology.

† Aided by a fellowship from the Dazian Foundation for Medical Research.

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referred to as the normal response. It is attributed to inhibition of elaboration or release of ACTH by prednisone (2–5). However, patients with Cushing's syndrome due to bilateral adrenocortical hyperfunction fail to respond in this manner to the prednisone suppression test (1).

In this clinic, for many years, the treatment of choice for non-tumorous Cushing's syndrome has consisted of pituitary irradiation alone or combined with unilateral adrenalectomy and has resulted in complete clinical remission in approximately 60 per cent of the patients (6). This study is concerned with evaluation of the results of the prednisone suppression test in patients with Cushing's syndrome prior to treatment and during partial or complete clinical remission following pituitary irradiation with or without unilateral adrenalectomy.

METHODS AND MATERIALS

The patients designated as normal subjects exhibited no demonstrable endocrine abnormalities. The patients with Cushing's syndrome had non-tumorous disease of the adrenal cortex. They had all manifested the classic clinical and laboratory features of the disease. Most of them had been surgically explored. They were divided into three categories: a) active untreated Cushing's syndrome, b) Cushing's syndrome in partial remission following unilateral adrenalectomy and pituitary irradiation, and c) Cushing's syndrome in complete remission following treatment with pituitary irradiation alone or combined with unilateral adrenalectomy. The laboratory and clinical data on these patients are presented in Table 1.

A control intramuscular gel-ACTH[1] test was performed initially in all patients. This test, as described previously by us (7, 8), involves the measurement of the free plasma 17-hydroxycorticoids before, and two hours following the intramuscular administration of 40 units of gel-ACTH. In normal subjects, the initial resting values range from 5–25 μg. per 100 ml. and the levels rise to 28–45 μg. per 100 ml. two hours following the administration of ACTH. In 3 previously reported patients with non-tumorous Cushing's syndrome, control levels of plasma 17-hydroxycorticoids were elevated; in 2 of these patients, adrenocortical hyper-responsiveness to gel-ACTH was demonstrated by rises in plasma 17-hydroxycorticoid levels to 67 and 70 μg. per 100 ml. respectively two hours following injection (8).

In this study, after the control determinations, both normal subjects and patients with adrenocortical hyperfunction were given 20 mg. of prednisone[2] twice daily by mouth for a period of seven days. Twelve to sixteen hours following the last dose of prednisone, an intramuscular gel-ACTH test was again performed. In some of the patients the dosage and duration of administration of prednisone was varied.

A suppressed response to ACTH following the administration of prednisone, as found in normal subjects, is arbitrarily represented by a plasma 17-hydroxycorticoid level of 26 μg. per 100 ml. or less two hours after the administration of gel-ACTH. Moreover, this value should be at least 10 μg. lower than a corresponding two-hour value obtained

[1] The Gel-ACTH was supplied through the courtesy of Organon, Inc., Orange, New Jersey.

[2] The prednisone (Meticorten) was supplied through the courtesy of Schering Corporation, Bloomfield, New Jersey.

TABLE 1. DATA ON 9 CASES OF NON-TUMOROUS CUSHING'S SYNDROME IN VARIOUS STAGES

Patients with non-tumorous Cushing's syndrome	Mode of previous therapy	Months from last therapy to time of study	Urinary 17-OH-corticoids* (mg./24 hrs.)	Plasma 17-OH-corticoids* (μg./100 ml.)	Urinary 17-keto-steroids* (mg./24 hrs.)	Clinical manifestations of Cushing's syndrome*
Group A—*active untreated*						
B.F.	None	—	17.0; 27.0	21.0	23.0	Hirsutism, obesity, hypertension, bruisability; menometrorrhagia. Diabetic glucose tolerance curve.
A.W.	None	—	11.0	28.0	16.0	Psychosis, buffalo hump, truncal obesity, bruisability, hypertension. Abnormal glucose tolerance curve.
Group B—*partial remission*						
L.S.	Irrad. of pit.† & unilat. adrenalectomy	5	17.0; 14.0	29.0	11.0	Obesity, plethora, moon-face.
R.W.	Irrad of pit.† & unilat. adrenalectomy	27	15.0; 13.0	30.0		Truncal obesity, hypertension, plethora, moon-face, bruisability.
L.M.	Irrad. of pit.‡ & unilat. adrenalectomy	5	14.0	20.0	11.0	Hypertension, amenorrhea, bruisability.
Group C—*complete remission*						
R.S.	Irrad. of pit.†	60	2.4	18.0	3.6	None
S.G.	Irrad. of pit.†	96	9.3	17.0	7.0	None
B.Fio	Irrad. of pit.†	14	6.6; 6.7	16.0	9.6	None
M.L.	Irrad. of pit.†	10	7.3	20.0	11.6	None

* At time of study.
† 4000 r delivered to the pituitary gland.
‡ Two courses of 4000 r each delivered to the pituitary gland.

prior to the administration of prednisone. Responses not conforming to this pattern are termed abnormal and indicate failure of suppression.

Plasma and urinary 17-hydroxycorticoids were determined by the method of Porter and Silber (9), and urinary 17-ketosteroids by the method of Holtorff and Koch (10).

RESULTS

Normal subjects (Fig. 1 and Table 2)

In 13 normal subjects administration of prednisone suppressed the adrenocortical response to gel-ACTH.

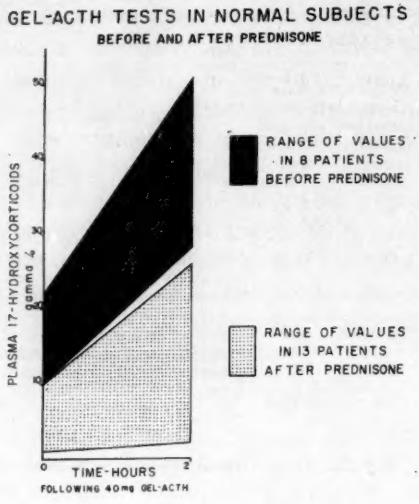

FIG. 1. *Normal subjects.* Effect of prednisone on the adrenocortical response to gel-ACTH, as measured by the level of plasma 17-hydroxycorticoids (μg./100 ml.).

TABLE 2. RESPONSE OF PLASMA 17-OH-CORTICOIDS TO INTRAMUSCULAR GEL-ACTH BEFORE AND AFTER PREDNISONE IN NORMAL SUBJECTS

Patient	Prednisone, oral daily dose (mg.)	Duration of therapy (days)	Plasma 17-OH-corticoids (μg./100 ml.)			
			Before prednisone		After prednisone	
			Before gel-ACTH	After gel-ACTH	Before gel-ACTH	After gel-ACTH
J.F.	40	7	22	51	7	11
T.L.	40	7	13	41	10	26
S.B.	40	7	13	43	1	22
J.O.	40	7	16	28	5	11
B.L.	40	7	12	39	6	14
J.V.	40	7	10	34	2	17
T.A.	40	7	16	32	4	14
A.M.	40	10	—	—	1	15
H.S.	40	20	17	35	9	13
W.B.	40	11	—	—	8	9
W.Z.	40	19	—	—	10	9
G.L.	40	32	—	—	3	2
M.S.	40	7	—	—	9	13

Cushing's syndrome. Group A—active, untreated (Fig. 2 and Table 1)

In 2 patients (B.F. and A.W.) there was no suppression of the response to gel-ACTH following the administration of 40 mg. of prednisone daily for seven days. Moreover, Patient B.F. responded no differently following the administration of 100 mg. of prednisone daily for seven days.

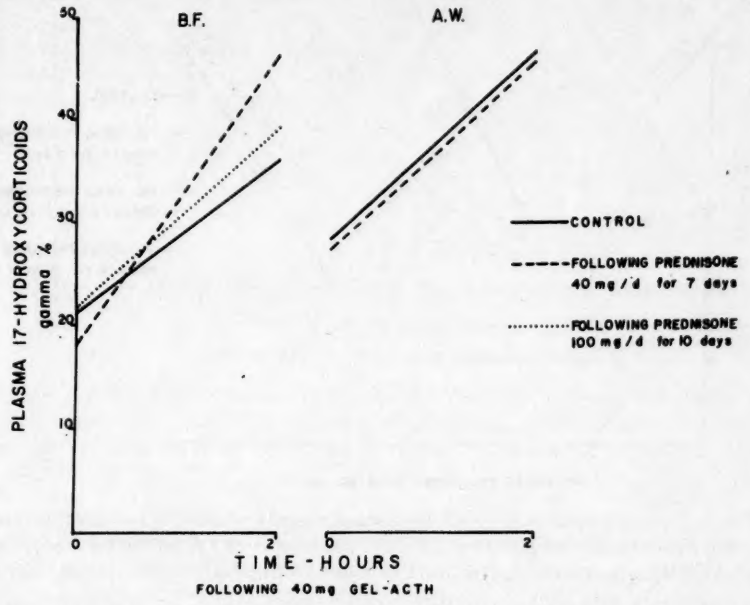

FIG. 2. *Cushing's syndrome. Group A—active, untreated.* Effect of prednisone on the adrenocortical response to gel-ACTH, as measured by the level of 17-hydroxycorticoids (μg./100 ml.).

Cushing's syndrome. Group B—partial remission following unilateral adrenalectomy and irradiation of the pituitary (Fig. 3 and Table 1)

Three patients were studied. One of these (L.M.) had persistent clinical and laboratory evidence of Cushing's syndrome following unilateral adrenalectomy and two courses of pituitary irradiation, each consisting of 4000 r delivered to the pituitary gland. In this patient, prednisone failed to suppress the response to gel-ACTH. In the second patient (R.W.), the administration of 40 mg. of prednisone daily both for seven and fourteen days resulted in only a partial suppression of the response to gel-ACTH. The third patient (L.S.) was tested five months following pituitary irradiation and unilateral adrenalectomy, and the result was similar to that in normal

subjects. At that time he still manifested obesity, some plethora, and some rounding of the facies, and the urinary excretion of 17-hydroxycorticoids was slightly elevated to 17 and 14 mg. per twenty-four hours.

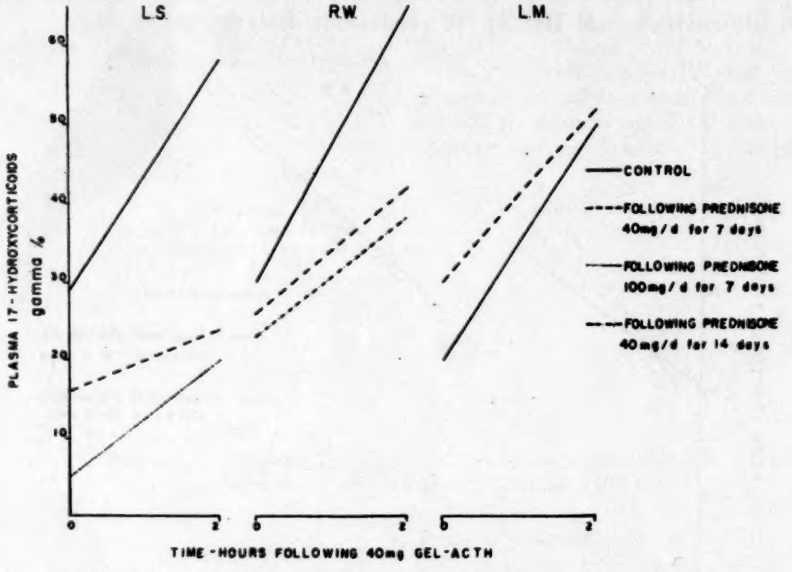

FIG. 3. *Cushing's syndrome. Group B—partial remission following unilateral adrenalectomy and irradiation of the pituitary.* Effect of prednisone on the adrenocortical response to gel-ACTH, as measured by the level of plasma 17-hydroxycorticoids (μg./100 ml.).

Cushing's syndrome. Group C—complete remission (Fig. 4 and Table 1)

Of 4 patients in this category, 2 (S.G. and R.S.) had experienced complete clinical and laboratory remission for five and eight years following pituitary irradiation. In both, the results of the prednisone suppression test were normal. In 2 other patients, complete clinical remission and a return to normal levels of plasma and urinary 17-hydroxycorticoids occurred following pituitary irradiation and unilateral adrenalectomy. However, an abnormal response to gel-ACTH was still noted in the prednisone suppression test when this was performed ten and fourteen months respectively following treatment.

DISCUSSION

These studies confirm the observation reported by Christy, Wallace and Jailer (1) that in patients with active non-tumorous Cushing's syndrome, as contrasted to normal subjects, adrenal responsiveness to corti-

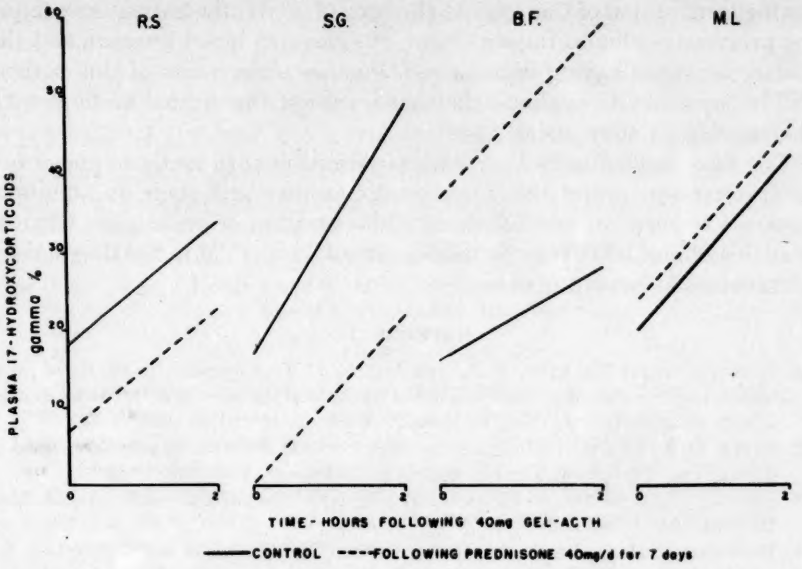

FIG. 4. *Cushing's syndrome. Group C—complete remission following irradiation of the pituitary alone or combined with unilateral adrenalectomy.* Effect of prednisone on the adrenocortical response to gel-ACTH, as measured by the level of plasma 17-hydroxycorticoids (μg./100 ml.).

cotropin is not suppressed following the administration of prednisone. We have extended these observations, and found persistence of an abnormal prednisone effect in 2 of 4 cases of non-tumorous Cushing's syndrome in complete clinical remission, as measured by clinical and laboratory criteria including normal levels of the plasma and urinary 17-hydroxycorticoids. Both of these patients have been well for almost a year following irradiation of the pituitary and unilateral adrenalectomy. However, in the 2 other cases of complete remission (similar criteria) lasting for five and eight years following only irradiation of the pituitary, the results of the prednisone test were normal. This difference may indicate that the abnormal prednisone effect observed in Cushing's syndrome does not return to normal as quickly as do other criteria.

In 2 of 3 patients in whom Cushing's syndrome was in partial remission following unilateral adrenalectomy and irradiation of the pituitary, results of the prednisone suppression test were abnormal. One of these 2 patients (L.M.) subsequently underwent a total adrenalectomy. In the third patient (L.S.) the results of the prednisone test were normal on two occasions. The tests in this patient were performed three to four months fol-

lowing termination of therapy. At the time of study, the patient was showing progressive clinical improvement, the elevated blood pressure and the violaceous striae having disappeared. Further observation of this patient will be necessary to evaluate the significance of the normal findings with the prednisone suppression test.

The fact that patients in a state of remission with neither clinical nor laboratory evidence of Cushing's syndrome may still show an abnormal response to corticotropin following administration of prednisone suggests that this abnormality may be used as an additional aid in the diagnosis of adrenocortical hyperfunction.

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THE EFFECT OF DIIODOHYDROXYQUIN (FLORAQUIN) VAGINAL SUPPOSITORIES ON THE LEVEL OF SERUM PROTEIN-BOUND IODINE*

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ABSTRACT

The effect of vaginally administered diiodohydroxyquin (Floraquin®) upon the concentration of serum protein-bound iodine (PBI) was studied in 10 women with Trichomonas vaginitis. The PBI levels were compared before, and on the twenty-eighth day of diiodohydroxyquin therapy; in 2 of the 10 patients a significant elevation was found. Thus, still another source of increased serum organic iodide concentration must be considered in evaluating the clinical significance of an elevated value for serum PBI.

DETERMINATION of the serum protein-bound iodine (PBI) concentration has become recognized as a valuable aid in the diagnosis of thyroid disease. A disadvantage of this measurement is the phenomenon of elevated PBI levels due to extraneous causes unrelated to thyroid function. The principal offenders in this regard have been the organic iodinated compounds, notably those used in diagnostic radiologic studies.

Diiodohydroxyquin (Floraquin®), an organic iodide compound, has been used extensively in the form of vaginal suppositories for the treatment of Trichomonas vaginitis. The question arose whether the vaginal administration of this material might alter the serum PBI level and thus be another source of diagnostic confusion. A brief study was therefore undertaken to evaluate this possibility.

MATERIALS AND METHODS

Ten patients with Trichomonas vaginalis infestation were selected. The diagnosis was established by both the clinical findings and the presence of large numbers of trichomonads in a wet smear of the vaginal secretions. Although all of the patients were parous, none was pregnant, and none had clinical evidence of thyroid disease. They ranged in age from 18 to 28 years. A blood sample for the determination of PBI concentration was obtained from each patient prior to the institution of therapy. Treatment consisted of

Received October 21, 1957.

* This investigation was supported in part by a grant from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service (PHS Grant A 565).

the insertion of a diiodohydroxyquin tablet twice daily into the vagina. Each tablet contained 100 mg. of diiodohyroxyquin (62 per cent iodine). The patients were also instructed to take a vaginal douche every other day using a warm dilute vinegar solution. Exactly four weeks after the start of treatment, each patient was again examined and another PBI determination was made. No other medications were given.

The serum PBI determinations were performed in duplicate by a modification of the method of Barker *et al.* (1). With this technic, the mean value for normal persons has been found to be 5.6 ± 1.2 μg. per 100 ml. Variations of less than 1 μg. per 100 ml. are attributed to the error inherent in the method.

RESULTS

Control PBI levels and those observed after four weeks of treatment are listed in Table 1. All the initial determinations were within the normal

TABLE 1. SERUM PROTEIN-BOUND IODINE LEVELS BEFORE AND AFTER VAGINAL DIIODOHYDROXYQUIN

Patient No.	Serum PBI (μg./100 ml.)		
	Initial	After therapy	Change
1	5.0	5.3	+0.3
2	5.4	5.4	0
3	4.7	5.1	+0.4
4	4.0	4.0	0
5	4.2	4.1	−0.1
6	5.6	5.9	+0.3
7	4.7	7.9*	+3.2
8	5.5	5.8	+0.3
9	4.2	7.1	+2.9
10	4.5	4.3	−0.2

* Four weeks after cessation of therapy, 7.6 μg./100 ml.

range, confirming the clinical impression of normal thyroid function. Eight of the 10 patients displayed no appreciable change in the serum PBI level after treatment, but in 2 patients (Nos. 7 and 9) there was a significant elevation after four weeks of treatment. In Patient 7 a third determination was made four weeks later. In the interval no treatment had been given. However, there was no significant change in the elevated PBI level.

The prescribed course of treatment involved administration of over 3 grams of iodine in organic combination over the four-week period. Retention of as little as 500 μg. distributed in the same extrathyroidal space as thyroid hormone would have been readily detected by a significant rise in the concentration of serum PBI. Diiodohydroxyquin is fairly insoluble and poorly absorbed when given orally (2). It is possible that the 2 patients

exhibiting an elevation of the PBI level after treatment had a more severe vaginitis with greater alteration of the vaginal mucosa, permitting greater absorption of the therapeutic agent. This explanation seems more probable than the alternate assumption that all of the patients absorbed significant amounts of the medication, but that the 8 in whom the PBI level remained normal metabolized the diiodohydroxyquin more readily than did the other 2 patients.

The results observed in this small number of cases suggest that in some instances enough diiodohydroxyquin is absorbed from the vagina to cause a moderate elevation in the concentration of serum PBI. If such a patient's pretreatment PBI level were near the upper limit of the normal range, the post-treatment level might well be such that a diagnosis of hyperthyroidism would be considered.

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20, and 21, 1958.*

The Committee on Local Arrangements—Dr. Roberto Escamilla, Chairman; Dr. Donald E. Bernstein, Dr. Peter H. Forsham, Dr. Minnie B. Goldberg, Dr. Gilbert S. Gordan, Dr. Francis S. Greenspan, Dr. C. H. Li, as members of the committee.

All Scientific Sessions will be held from 9:00 A.M. to 5:00 P.M. daily in the Colonial Ballroom, and in addition there will be simultaneous afternoon sessions in the Italian Room. The annual dinner is scheduled for Friday, June 20 at 7:30 P.M., preceded by cocktails at 6:30 P.M.

All reservations for rooms must be made through the AMA Housing Bureau, Room 300, 61 Grove Street, San Francisco 2, California. If possible, official application forms as published in the Journal of the American Medical Association should be used. Arrival and departure dates must be stated. All requests must be accompanied by check or money order (not cash) made payable to "The AMA Housing Bureau" in the amount of $10.00 per room, which will be credited to your hotel account. Be sure to state your first, second and third choice of hotels. It is important to make reservations immediately in order to avoid disappointment.

Final program, membership card and advance registration forms will be sent on May 15, 1958 to members whose current dues have been paid.

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1958 ANNUAL MEETING

The next Annual Meeting of The American Goiter Association will be held in the St. Francis Hotel, San Francisco, California, on *June 17, 18 and 19, 1958.*

All reservations for rooms (accompanied by a $10 deposit) must be made through the AMA Housing Bureau, Room 300, 61 Grove Street, San Francisco 2, California.

AMERICAN GERIATRICS SOCIETY— THE 1958 ANNUAL MEETING

This will be the First Annual Conference on "Clinical Nutrition in Relation to the Aging Process," sponsored by the American Geriatrics Society, the Gerontological Society, the Institute for Metabolic Research and the Committee on Graduate Medical Education of the Alameda-Contra Costa Medical Association. Eminent men from this country and abroad will be on the Faculty.

The meeting will be held in the Main Auditorium of the Highland Alameda County Hospital, 2701 Fourteenth Avenue, Oakland 6, California, on *June 18 and 19, 1958.*

RICHARD J. KRAEMER, M.D.,
Secretary-Treasurer,
2907 Post Road,
Greenwood, Rhode Island

REFRESHER COURSES IN PEDIATRICS

The Children's Hospital of Philadelphia and the Graduate and Undergraduate Schools of Medicine, University of Pennsylvania, announce a series of short refresher courses for practitioners, pediatricians and clinical pathologists. These courses will emphasize developments of the past few years which are important to the physician in practice. There will be panel discussions, demonstrations, conferences and case presentations. *Course 1.* "Pediatric Advances"; May 26 through 30, 1958; tuition $115.00. *Course 2.* "Practical Pediatric Hematology"; June 2, 3 and 4, 1958; tuition $75.00. *Course 3.* "Hemolytic Disease of the Newborn"; June 5 and 6, 1958; tuition $50.00. Applications and correspondence should be forwarded to Irving J. Wolman, M.D., Children's Hospital of Philadelphia, 1740 Bainbridge Street, Philadelphia 46, Pa.

REPRINTS WANTED ON ENDOCRINE-CANCER RELATIONSHIPS

The Cancer Chemotherapy National Service Center is seeking reprints for a reference collection covering the broadest range of endocrine-cancer relationships, as well as basic endocrinology. In particular it needs clinical data on cancer patients treated with hormones, data on the biological activities of hormones, analogs and antagonistic substances, and data on the effects of hormones on animal tumors. Assay data on steroids would be especially welcome. Those wishing to assist, please send reprints to: Dr. D. Jane Taylor, Endocrinology Section, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda 14, Maryland.

SOCIETY FOR THE SCIENTIFIC STUDY OF SEX

The Society for the Scientific Study of Sex will hold its first annual meeting on *November 8, 1958*, at the Barbizon Plaza Hotel in New York City. For details, write to Robert V. Sherwin, 1 East 42nd Street, New York 17, N. Y.

INTER-SOCIETY CYTOLOGY COUNCIL

The Annual Scientific Meeting of the Inter-Society Cytology Council will be held in the Hotel Statler, New York, N. Y., on *November 13, 14 and 15, 1958*. Paul F. Fletcher, M.D., Secretary, 634 North Grand Avenue, St. Louis 3, Missouri.

THIRD WORLD CONGRESS ON FERTILITY AND STERILITY

The Third World Congress on Fertility and Sterility, sponsored by the International Fertility Association, will be held in Amsterdam, Holland, *June 7–13, 1959*. Registration fee: full member U.S. $40 (after Jan. 1, 1959, $50); accompanying family members, U.S. $15. Titles for papers should be sent *not later than June 1958*, to both Dr. Alfonso Alvarez-Bravo, Avenida Horacio 1506, Mexico City 5, D. F., Mexico, (Chairman); and Prof. B. S. ten Berge, Academisch Ziekenhuis, Groningen, Holland (executive member of the Program Committee). For further information and registration, apply to Dr. L. I. Swaab, Hon. Secretary Third World Congress on Fertility and Sterility, Sint Agnietenstraat 4, Amsterdam-C, Holland, or to the nearest Wagons Lits/Cook office.